WASTEWATER TREATMENT
WATER QUALITY

PROFESSIONAL DEVELOPMENT COURSE
4 CEUs, 40 PDHs, 40 Training Hours or Contact Hours

Technical Learning College

Printing and Saving Instructions

The best thing to do is to download this pdf document to your computer desktop and open it with Adobe Acrobat reader.

Abode Acrobat reader is a free computer software program and you can find it at Abode Acrobat's website.

You can complete the course by viewing the course materials on your computer or you can print it out. We give you permission to print this document.

Printing Instructions: If you are going to print this document, this document is designed to be printed double-sided or duplexed but can be single-sided.

This course booklet does not have the assignment. Please visit our website and download the assignment also.

Hyperlink to the assignment
http://www.tlch2o.com/PDF/WWTWaterQuality%20Assignment.pdf

State Approval Listing Link, check to see if your State accepts or has pre-approved this course. Not all States are listed. Not all courses are listed. If the course is not accepted for CEU credit, we will give you the course free if you ask your State to accept it for credit.

Professional Engineers: Most states will accept our courses for credit but we do not officially list the States or Agencies.

State Approval Listing URL…
http://www.tlch2o.com/PDF/CEU%20State%20Approvals.pdf

You can obtain a printed version from TLC for an additional $79.95 plus shipping charges.

Contributing Editors
Joseph Camerata has a BS in Management with honors (magna cum laude). He retired as a Chemist in 2006 having worked in the field of chemical, environmental, and industrial hygiene sampling and analysis for 40 years. He has been a professional presenter at an EPA analytical conference at the Biosphere in Arizona and a presenter at an AWWA conference in Mesa, Arizona. He also taught safety classes at the Honeywell and City of Phoenix, and is a motivational/inspirational speaker nationally and internationally.

Eric Pearce S.M.E., chemistry and biological review.

Pete Greer S.M.E., retired biology instructor.

Jack White, Environmental, Health, Safety expert, City of Phoenix. Art Credits.

Technical Learning College's Scope and Function

Technical Learning College (**TLC**) offers affordable continuing education for today's working professionals who need to maintain licenses or certifications. TLC holds approximately 80 different governmental approvals for granting of continuing education credit.

TLC's delivery method of continuing education can include traditional types of classroom lectures and distance-based courses or independent study. Most of TLC's distance based or independent study courses are offered in a print-based format and you are welcome to examine this material on your computer with no obligation. Our courses are designed to be flexible and for you to finish the material at your leisure. Students can also receive course materials through the mail. The CEU course or e-manual will contain all your lessons, activities and assignments. Most CEU courses allow students to submit lessons using e-mail or fax, however some courses require students to submit lessons by postal mail (See the course description for more information). Students have direct contact with their instructor—primarily by e-mail. TLC's CEU courses may use such technologies as the World Wide Web, e-mail, CD-ROMs, videotapes and hard copies (See the course description). Make sure you have access to the necessary equipment before enrolling, i.e., printer, Microsoft Word and/or Adobe Acrobat Reader. Some courses may require proctored exams, depending upon your state requirements.

Flexible Learning
At TLC, there are no scheduled online sessions you need contend with, nor are you required to participate in, learning teams or groups designed for the "typical" younger campus-based student. You will work at your own pace, completing assignments in time frames that work best for you. TLC's method of flexible individualized instruction is designed to provide each student the guidance and support needed for successful course completion.

We will beat any other training competitor's price for the same CEU material or classroom training. Student satisfaction is guaranteed.

Course Structure
TLC's online courses combine the best of online delivery and traditional university textbooks. Online you will find the course syllabus, course content, assignments, and online open book exams. This student-friendly course design allows you the most flexibility in choosing when and where you will study.

Classroom of One
TLC Online offers you the best of both worlds. You learn on your own terms and your own time, but you are never on your own. Once you enrolled, you will be assigned a personal student service representative who works with you on an individualized basis throughout your program of study. Course-specific faculty members are assigned at the beginning of each course, providing the academic support you need to successfully complete each course.

Satisfaction Guaranteed
Our iron-clad, risk-free Guarantee ensures you will be another satisfied TLC student.

We have many years of experience, dealing with thousands of students. We assure you, our customer satisfaction is second to none. This is one reason we have taught more than 10,000 students.

Our administrative staff is trained to provide the best customer service in town. Part of that training is knowing how to solve most problems on the spot with an exchange or refund.

TLC Continuing Education Course Material Development
TLC's continuing education course material development was based upon several factors; extensive academic research, advice from subject matter experts, data analysis, task analysis and training needs assessment process information gathered from other states.

Please fax or e-mail the answer key to TLC
Western Campus Fax (928) 272-0747.

Rush Grading Service
If you need this assignment graded and the results mailed to you within a 48-hour period, prepare to pay an additional rush service handling fee of $50.00. This fee may not cover postage costs. If you need this service, simply write RUSH on the top of your Registration Form. We will place you in the front of the grading and processing line.

For security purposes, please fax or e-mail a copy of your driver's license and always call us to confirm we've received your assignment and to confirm your identity.

Thank you…

Course Description

Wastewater Treatment Water Quality CEU Training Course

This is a review of various and complex wastewater treatment methods, water quality, sampling techniques, bug identification, disinfection, sludge disposal and related WWT subjects. This course is general in nature and not state specific but will contain different wastewater treatment, activated sludge methods and wastewater quality, permit writing methods, sampling policies and ideas. You will not need any other materials for this course.

This course will cover various wastewater treatment methods including:

- Best conventional pollutant control technology (BCT) for conventional pollutants and applicable to existing dischargers.
- Best practicable control technology currently available (BPT) for conventional, toxic and non-conventional pollutants and applicable to existing dischargers.
- Best available technology economically achievable (BAT) for toxic and non-conventional pollutants and applicable to existing dischargers.
- New source performance standards (NSPS) for conventional pollutants and applicable to new sources.

Intended Audience

Wastewater Treatment Operators; Pretreatment and Industrial Waste Inspectors. The target audience for this course is the person interested in working in a wastewater treatment or pretreatment/industrial wastewater facility and/or wishing to maintain CEUs for certification license or to learn how to do the job safely and effectively, and/or to meet education needs for promotion.

Prerequisites: None

Course Procedures for Registration and Support

All of TLC's correspondence courses have complete registration and support services offered. Delivery of services will include e-mail, web site, telephone, fax and mail support. TLC provides immediate and prompt service.

When a student registers for a correspondence course, he or she is assigned a start date and an end date. It is the student's responsibility to note dates for assignments and keep up with the course work. If a student falls behind, he or she must contact TLC and request an end date extension in order to complete the course. It is the prerogative of TLC to decide whether to grant the request.

All students will be tracked by their social security number or a unique number assigned to the student.

Instructions for Written Assignments

The Wastewater Treatment Water Quality CEU training distance learning course uses a fill-in-the-blank style answer key. You can write your answers in this manual or type out your own answer key. TLC would prefer that you type out and e-mail final assignment to TLC, but it is not required.

Feedback Mechanism (examination procedures)
Each student will receive a feedback form as part of the study packet. You will be able to find this form in the rear of the course or lesson.

Security and Integrity
All students are required to do their own work. All lesson sheets and final exams are not returned to the student to discourage and sharing of answers. Any fraud or deceit and the student will result in forfeiture of all fees, and the appropriate agency will be notified.

Grading Criteria
TLC will offer the student either pass/fail or a standard letter grading assignment. If TLC is not notified, you will only receive a pass/fail notice.

Required Texts
The Wastewater Treatment Water Quality CEU training course will not require any other materials. This course comes complete. No other materials are needed.

Environmental Terms, Abbreviations, and Acronyms
TLC provides a glossary that defines, in non-technical language, commonly used environmental terms appearing in publications and materials. It also explains abbreviations and acronyms used throughout EPA and other agencies. You can find the glossary in the rear of the manual.

Recordkeeping and Reporting Practices
TLC will keep all student records for a minimum of five years. It is your responsibility to give the completion certificate to the appropriate agencies. TLC will mail a copy to Indiana, Pennsylvania, Texas, or any other state that requires a copy from the Training Provider.

ADA Compliance
TLC will make reasonable accommodations for persons with documented disabilities. Students should notify TLC and their instructors of any special needs. Course content may vary from this outline to meet the needs of this particular group. There is an alternative assignment available.

The final grade options are as follows:
Letter grade (**A**, **B**, **C**, **D**, **F**) - These grades are awarded based on the course grading scale.
Withdrawn (**W** or **Y**) - Students who enroll but do not participate in the class may withdraw themselves by calling Admissions and Records, or their instructor may withdraw them. Either case will result in a grade of "**W**." Note that participation means the completion of a single homework assignment or an exam. Completion of the pretest and/or syllabus receipt does not imply course participation.

Credit/no credit option (**P**/**Z**) - None Available

Note to students: Keep a copy of everything you submit. That way if your work is lost you can submit your copy for grading. If you do not receive your graded assignment or quiz results within two or three weeks after submitting it, please contact your instructor.

We expect every student to produce his/her original, independent work. Any student whose work indicates a violation of the Academic Misconduct Policy (cheating, plagiarism) can expect penalties as specified in the Student Handbook, which is available through Student Services; contact them at (928) 468-0665.

A student who registers for a Distance Learning course is assigned a **"start date"** and an **"end date."** It is the student's responsibility to note due dates for assignments and to keep up with the course work.

If a student falls behind, she or he must contact the instructor and request an extension of her/his ***end date*** in order to complete the course. It is the prerogative of the instructor to decide whether or not to grant the request. You will have 90 days from receipt of this manual to complete it in order to receive your Continuing Education Units (**CEUs**) or Professional Development Hours (**PDHs**). A score of 70% or better is necessary to pass this course.

If you need any assistance, please email all concerns to info@tlch2o.com.

Educational Mission
The educational mission of TLC is:

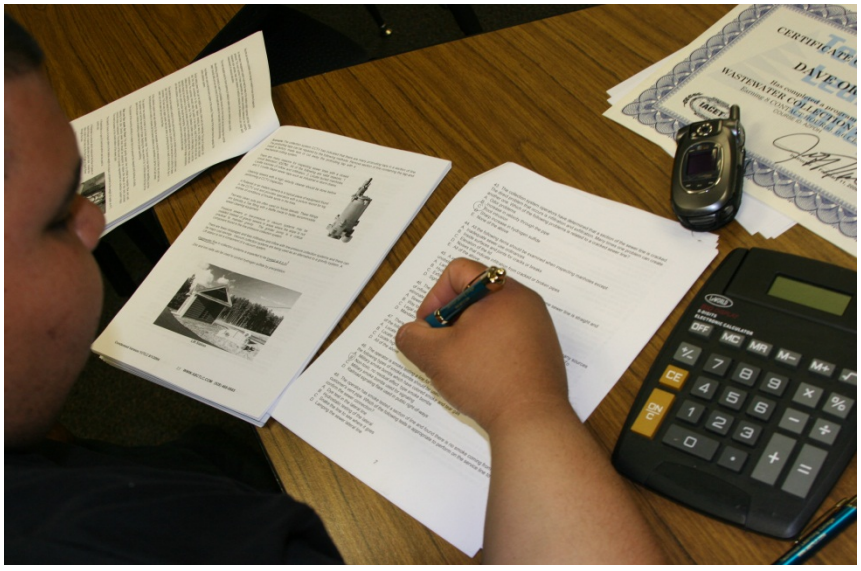
To provide TLC students with comprehensive and ongoing training in the theory and skills needed for the environmental education field,

To provide TLC students opportunities to apply and understand the theory and skills needed for operator certification,

To provide opportunities for TLC students to learn and practice environmental educational skills with members of the community for the purpose of sharing diverse perspectives and experience,

To provide a forum in which students can exchange experiences and ideas related to environmental education,

To provide a forum for the collection and dissemination of current information related to environmental education, and to maintain an environment that nurtures academic and personal growth.



Course Objective: To provide a detailed understanding in effective and efficient wastewater treatment and disinfection methods including activated sludge methods and generally accepted wastewater treatment sampling techniques and biological monitoring, bug identification and microorganism control methods.

Important Information about this Manual

This manual has been prepared to educate employees in the general awareness of dealing with complex wastewater treatment procedures and regulatory requirements for safely handling hazardous and toxic materials. The scope of the problem is quite large, requiring a major effort to bring it under control. Employee health and safety, as well as that of the public, depend upon careful application of safe treatment procedures. The manner in which we deal with such hazards will affect the earth and its inhabitants for many generations to come.

This manual will cover general laws, regulations, required procedures and generally accepted policies relating to wastewater treatment and wastewater sampling. It should be noted, however, that the regulation of wastewater treatment, sampling and other hazardous materials is an ongoing process and subject to change over time. For this reason, a list of resources is provided to assist in obtaining the most up-to-date information on various subjects.

This manual is not a guidance document for employees who are involved with pollution control or wastewater treatment. It is not designed to meet the requirements of the United States Environmental Protection Agency (EPA) or Department of Labor-Occupational Safety and Health Administration (OSHA) or state environmental or health departments.

This course manual will provide general educational awareness guidance of activated sludge. This document is not a detailed wastewater treatment textbook or a comprehensive source book on occupational safety and health.

Technical Learning College or Technical Learning Consultants, Inc. makes no warranty, guarantee or representation as to the absolute correctness or appropriateness of the information in this manual and assumes no responsibility in connection with the implementation of this information. It cannot be assumed that this manual contains all measures and concepts required for specific conditions or circumstances. This document should be used for educational guidance and is not considered a legal document.

Individuals who are responsible for the treatment of wastewater, wastewater sampling or the health and safety of workers at wastewater sites should obtain and comply with the most recent federal, state, and local regulations relevant to these sites and are urged to consult with OSHA, EPA and other appropriate federal, state, health and local agencies.

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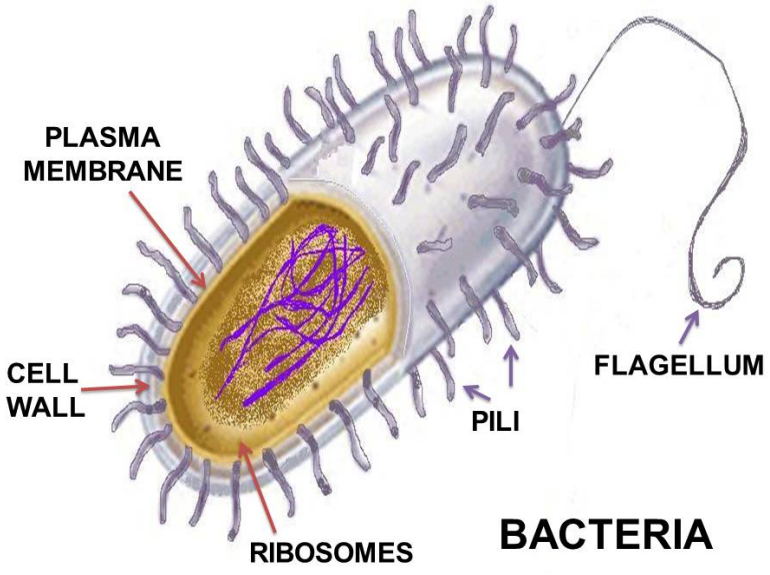
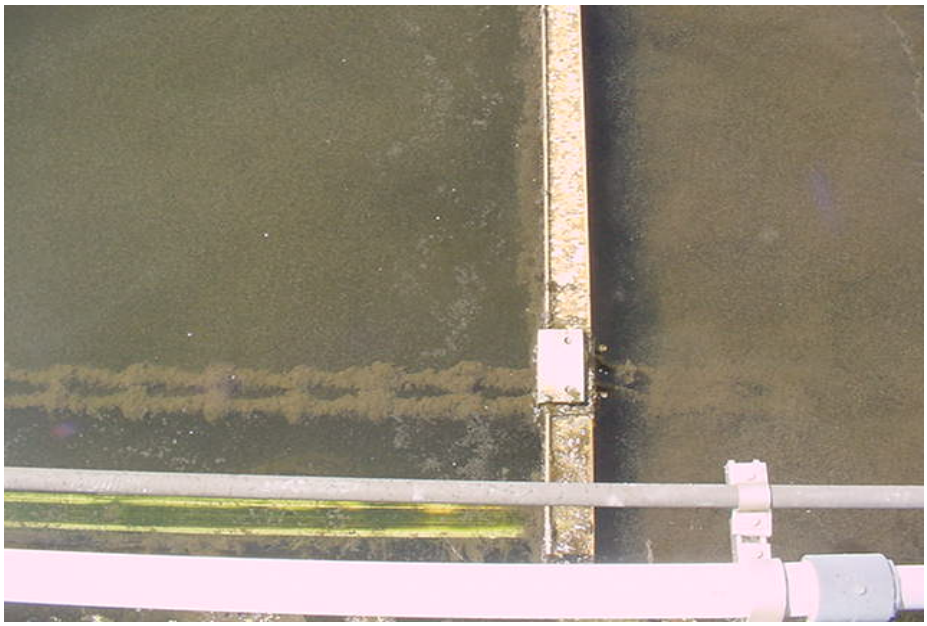


Common Wastewater Acronyms and Terms

Acronyms and Abbreviations
A/E Contract: Architectural and Engineering Contracts
A/O: Pho-redox
AMSA: Association of Metropolitan Sewerage Agencies
AOB: Ammonia Oxidizing Bacteria
ASM: Activated Sludge Model
AT3: Aeration Tank 3
BABE: Bio-Augmentation Batch Enhanced
BAF: Biological Aerated Filter
BAR: Bio-Augmentation Regeneration/Reaeration
BCFS: Biological Chemical Phosphorus and Nitrogen Removal
bDON: Biodegradable Fraction of Dissolved Organic Nitrogen
BHRC: Ballasted High Rate Clarification Processes
BNR: Biological Nutrient Removal
BOD: Biochemical Oxygen Demand
BOD5: Biochemical Oxygen Demand (5-day)
BPR: Biological Phosphorus Removal
COD: Chemical Oxygen Demand
CSO: Combined Sewer Overflow
CWA: Clean Water Act
CWSRF: Clean Water State Revolving Fund
D&D: Drying and Dewatering Facility
DAF: Dissolved Air Flotation
DNR: Department of Natural Resources
DO: Dissolved Oxygen
DON: Dissolved Organic Nitrogen
E1: Estrone
E2:17 ß-estradiol
EBPR: Enhanced Biological Phosphorus Removal
EDC : Endocrine Disrupting Chemicals
EDTA: Ethylene Diamine Tetraacetic Acid
EE2: 17α-ethynylestradiol
EPA: U.S. Environmental Protection Agency
EPA or USEPA: United States Environmental Protection Agency
FFS: Fixed-film Systems
FWPCA : Federal Water Pollution Control Act
FWS: Free Water Surface
GAO: Glycogen Accumulating Organism
GIS: Geographic Information System
HHWP: Household Hazardous Waste Collection Program
HRSD: Hampton Roads Sanitation District
HRT: Hydraulic Retention Time
I&C: Instrumentation and Control System
I/I: Infiltration and Inflow
iDON: Inert Dissolved Organic Nitrogen
ISF: Intermittent Sand Filter
ISS: Inline Storage System
IWA: International Water Association

IWPP: Industrial Waste Pretreatment Program
JHB: Johannesburg
LIMS: Laboratory Information Management Systems
MAUREEN: Mainstream Autotrophic Recycle Enhanced N-removal
MBBR: Moving-Bed Biofilm Reactor
MBDT: Minority Business Development and Training
MBE: Minority Business Enterprise
MBR: Membrane Bioreactor
MGD: Million Gallons per Day
MLE: Modified Ludzack Ettinger
MUCT : Modified University of Capetown
N: Nitrogen
NOAA : National Oceanic and Atmospheric Administration
NOB : Nitrite Oxidizing Bacteria
NPDES : National Pollutant Discharge Elimination System
NTT: Nitrogen Trading Tool
ORD: EPA Office of Research and Development
ORP: Oxidation Reduction Potential
OWASA : Orange Water and Sewer Authority
OWM : EPA Office of Wastewater Management
P: Phosphorus
P2: Pollution Prevention Initiative
PAH: Polycyclic Aromatic Hydrocarbons
PAO: Phosphate Accumulating Organism
PHA: Polyhydroxyalkanoates
PHB: Poly-B-hydroxy-butyrate
PHV: Poly-hydroxy valerate
POTW : Publicly Owned Treatment Works
PPCPs : Pharmaceuticals and Personal Care Products
QA/QC: Quality Assurance and Quality Control
RAS: Return Activated Sludge
RBC: Rotating Biological Contactor
rbCOD: Readily Biodegradable Chemical Oxygen Demand
rDON: Recalcitrant Dissolved Organic Nitrogen
RO: Reverse Osmosis
RSF: Recirculating Sand Filters
S/W/MBE: Small, Women's, Minority Business Enterprise
SAV: Submerged Aquatic Vegetation
SBR : Sequencing Batch Reactors
SHARON : Single Reactor High-activity Ammonia Removal Over Nitrite
SND : Simultaneous Nitrification-Denitrification
SRT : Solids Retention Time
SSES: Sewer System Evaluation Survey
SSO: Sanitary Sewer Overflow
STAC: Chesapeake Bay Program Scientific and Technical Advisory Committee
SWIS: Subsurface Wastewater Infiltration System
TAT: Technical Advisory Team
TDS: Total Dissolved Solids
TKN: Total Kjeldahl Nitrogen
TMDL :Total Maximum Daily Loads
TN: Total Nitrogen

TP: Total Phosphorus
TSS : Total Suspended Solids
TUDP: Bio-P Model of the Delft University of Technology
UCT: University of Capetown
USDA: U.S. Department of Agriculture
USGS: U.S. Geological Survey
VFA: Volatile Fatty Acids
VIP: Virginia Initiative Plant
VSS: Volatile Suspended Solids
WAS: Waste Activated Sludge
WEF: Water Environment Federation
WERF: Water Environment Research Foundation
WPAP: Water Pollution Abatement Program
WQS: Water Quality Standard
WWTP: Wastewater Treatment Plant



Rectangular clarifier mechanism, flights, and chains.

Here is one of TLC's professors Marcos Aparecido Silva Bueno showing microscopic views of commonly found MO's in a classroom setting. Professor Marcos Aparecido Silva Bueno is a world renowned microbiological expert.

WWT Key Words

This glossary includes a collection of terms used in this course and an explanation of each term.

Act or "the Act" *[40 CFR §403.3(b)]*
The Federal Water Pollution Control Act, also known as the Clean Water Act, as amended, 33 USC 1251*et.seq.*

Approval Authority *[40 CFR §403.3(c)]*
The Director in an NPDES State with an approved State Pretreatment Program and the appropriate EPA Regional Administrator in a non-NPDES State or State without an approved pretreatment program.

Approved POTW Pretreatment Program or Program *[40 CFR §403.3(d)]*
A program administered by a POTW that meets the criteria established in 40 CFR Part 403 and which has been approved by a Regional Administrator or State Director.

Approved State Pretreatment Program
A program administered by a State that meets the criteria established in 40 CFR §403.10 and which has been approved by a Regional Administrator

Approved/Authorized State
A State with an NPDES permit program approved pursuant to section 402(b) of the Act and an approved State Pretreatment Program.

Baseline Monitoring Report (BMR) *[paraphrased from 40 CFR §403.12(b)]*
A report submitted by categorical industrial users (CIUs) within 180 days after the effective date of an applicable categorical standard, or at least 90 days prior to commencement of discharge for new sources, which contains specific facility information, including flow and pollutant concentration data. For existing sources, the report must also certify as to the compliance status of the facility with respect to the categorical standards.

Best Available Technology Economically Achievable (BAT)
A level of technology based on the best existing control and treatment measures that are economically achievable within the given industrial category or subcategory.

Best Management Practices (BMPs)
Schedules of activities, prohibitions of practices, maintenance procedures, and other management practices to prevent or reduce the pollution of waters of the U.S. BMPs also include treatment requirements, operating procedures and practices to control plant site runoff, spillage or leaks, sludge or waste disposal, or drainage from raw material storage.

Best Practicable Control Technology Currently Available (BPT)
A level of technology represented by the average of the best existing wastewater treatment performance levels within an industrial category or subcategory.

Best Professional Judgment (BPJ)
The method used by a permit writer to develop technology-based limitations on a case-by-case basis using all reasonably available and relevant data.

Blowdown
The discharge of water with high concentrations of accumulated solids from boilers to prevent plugging of the boiler tubes and/or steam lines. In cooling towers, blowdown is discharged to reduce the concentration of dissolved salts in the recirculating cooling water.

Bypass *[40 CFR §403.17(a)]*
The intentional diversion of waste streams from any portion of an Industrial User's treatment facility.

Categorical Industrial User (CIU)
An industrial user subject to National categorical pretreatment standards.

Categorical Pretreatment Standards *[40 CFR § 403.6 and 40 CFR Parts 405-471*]
Limitations on pollutant discharges to POTWs promulgated by the EPA in accordance with Section 307 of the Clean Water Act, that apply to specific process wastewater discharges of particular industrial categories.

Chain of Custody (COC)
A record of each person involved in the possession of a sample from the person who collects the sample to the person who analyzes the sample in the laboratory.

Chronic
A stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. Chronic should be considered a relative term depending on the life span of an organism. The measurement of chronic effect can be reduced growth, reduced reproduction, etc., in addition to lethality.

Clean Water Act (CWA)
The common name for the Federal Water Pollution Control Act. Public law 92-500; 33 U.S.C. 1251 et seq.; legislation which provides statutory authority for both NPDES and Pretreatment Programs.

Code of Federal Regulations (CFR)
A codification of Federal rules published annually by the Office of the Federal Register National Archives and Records Administration. Title 40 of the CFR contains the regulations for *Protection of the Environmen*t.

Combined Sewer Overflow (CSO)
A discharge of untreated wastewater from a combined sewer system at a point prior to the headworks of a publicly owned treatment works. CSOs generally occur during wet weather (rainfall or snowfall). During periods of wet weather, these systems become overloaded, bypass treatment works, and discharge directly to receiving waters.

Combined Wastestream Formula (CWF) *[paraphrased from 40 CFR §403.6(e)]*
Procedure for calculating alternative discharge limits at industrial facilities where a regulated wastestream from a categorical industrial user is combined with other waste streams prior to treatment.

Compliance Schedule
A schedule of remedial measures included in a permit or an enforcement order, including a sequence of interim requirements (for example, actions, operations, or milestone events) that lead to compliance with the CWA and regulations.

Composite Sample
Sample composed of two or more discrete samples. The aggregate sample will reflect the average water quality covering the compositing or sample period.

Concentration-based Limit
A limit based upon the relative strength of a pollutant in a wastestream, usually expressed in mg/l.

Continuous Discharge
A discharge that occurs without interruption during the operating hours of a facility, except for infrequent shutdowns for maintenance, process changes or similar activities.

Control Authority *[paraphrased from 40 CFR § 403.12(a)]*
A POTW with an approved pretreatment program or the approval authority in the absence of a POTW pretreatment program.

Conventional Pollutants
BOD, TSS, fecal coliform, oil and grease, and pH

Daily Maximum Limitations
The maximum allowable discharge of pollutants during a 24-hour period. Where daily maximum limitations are expressed in units of mass, the daily discharge is the total mass discharged over the course of the day. Where daily maximum limitations are expressed in terms of a concentration, the daily discharge is the arithmetic average measurement of the pollutant concentration derived from all measurements taken that day.

Detection Limit
The minimum concentration of an analyte (substance) that can be measured and reported with a 99% confidence that the analyte concentration is greater than zero as determined by the procedure set forth in 40 CFR Part 136, Appendix B.

Development Document
Detailed report of studies conducted by the U.S. EPA for the purpose of establishing effluent guidelines and categorical pretreatment standards.

Dilute Wastestream *[paraphrased from 40 CFR §403.6(e)(1)(i)]*
For purposes of the combined wastestream formula, the average daily flow (at least a 30-day average) from :(a) boiler blowdown streams, non-contact cooling streams, storm water streams, and demineralized backwash streams; provided, however, that where such streams contain a significant amount of a pollutant, and the combination of such streams, prior to treatment, with an industrial user's regulated process wastestream(s) will result in a substantial reduction of that pollutant, the Control Authority, upon application of the industrial user, may exercise its discretion to determine whether such stream(s) should be classified as diluted or unregulated. In its application to the Control Authority, the industrial user must provide engineering, production, sampling and analysis, and such other information so the control authority can make its determination; or (b) sanitary wastestreams where such streams are not regulated by a categorical pretreatment standard; or (c) from any process wastestreams which were, or could have been, entirely exempted from categorical pretreatment standards pursuant to paragraph 8 of the NRDC v. Costle Consent Decree (12 ERC 1833) for one more of the following reasons (see Appendix D of 40 CFR Part 403):
a. the pollutants of concern are not detectable in the effluent from the industrial user (paragraph(8)(a)(iii));
b. the pollutants of concern are present only in trace amounts and are neither causing nor likely to cause toxic effects (paragraph (8)(a)(iii));
c. the pollutants of concern are present in amounts too small to be effectively deduced by technologies known to the Administrator (paragraph (8)(a)(iii)); or
d. the wastestream contains only pollutants which are compatible with the POTW (paragraph (8)(b)(I)).

Effluent Limitations Guideline
Any effluent limitations guidelines issued by the EPA pursuant to Section 304(b) of the CWA. These regulations are published to adopt or revise a national standard prescribing restrictions on quantities, rates, and concentrations of chemical, physical, biological, and other constituents which are discharged from point sources, in specific industrial categories (e.g., metal finishing, metal molding and casting, etc.).

Enforcement Response Plan *[paraphrased from 40 CFR §403.8(f)(5)]*
Step-by-step enforcement procedures followed by Control Authority staff to identify, document, and respond to violations.

Existing Source
Any source of discharge, the construction or operation of which commenced prior to the publication by the EPA of proposed categorical pretreatment standards, which will be applicable to such source if the standard is thereafter promulgated in accordance with Section 307 of the Act.

Federal Water Pollution Control Act (FWPCA)
The title of Public law 92-500; 33 U.S.C. 1251 et seq., also known as the Clean Water Act (CWA),enacted October 18, 1972.

Flow Weighted Average Formula (FWA) *[paraphrased from 40 CFR §403.6(e)]*
A procedure used to calculate alternative limits where wastestreams regulated by a categorical pretreatment standard and nonregulated wastestreams combine after treatment but prior to the monitoring point.

Flow Proportional Composite Sample
Combination of individual samples proportional to the flow of the wastestream at the time of sampling.

Fundamentally Different Factors *[paraphrased from 40 CFR §403.13]*
Case-by-case variance from categorical pretreatment standards based on the factors considered by the EPA in developing the applicable category/subcategory being fundamentally different than factors relating to a specific industrial user.

General Prohibitions *[40 CFR §403.5(a)(1)]*
No user shall introduce into a POTW any pollutant(s) which cause pass through or interference.

Grab Sample
A sample which is taken from a wastestream on a one-time basis with no regard to the flow of the wastestream and without consideration of time. A single grab sample should be taken over a period of time not to exceed 15 minutes.

Indirect Discharge or Discharge *[40 CFR §403.3(g)]*
The introduction of pollutants into a POTW from any non-domestic source regulated under section 307(b), (c), or (d) of the Act.

Industrial User (IU) or User *[40 CFR §403.3(h)]*
A source of indirect discharge.

Industrial Waste Survey
The process of identifying and locating industrial users and characterizing their industrial discharge.

Inhibition Concentration
Estimate of the toxicant concentration that would cause a given percent reduction (e.g., IC25) in a nonlethal biological measurement of the test organisms, such as reproduction or growth.

Interference *[paraphrased from 40 CFR §403.3(i)]*
A discharge which, alone or in conjunction with a discharge or discharges from other sources, both: (1)inhibits or disrupts the POTW, its treatment processes or operations, or its sludge processes, use or disposal; and (2) therefore is a cause of a violation of any requirement of the POTW's NPDES permit (including an increase in the magnitude or duration of a violation) or of the prevention of sewage sludge use or disposal in compliance with ... [applicable] statutory provisions and regulations or permits issued there under (or more stringent State or local regulations)

Local Limits *[paraphrased 40 CFR § 403.5(c)]*
Specific discharge limits developed and enforced by POTWs upon industrial or commercial facilities to implement the general and specific discharge prohibitions listed in 40 CFR §§403.5(a)(1) and (b).

Monthly Average
The arithmetic average value of all samples taken in a calendar month for an individual pollutant parameter. The monthly average may be the average of all grab samples taken in a given calendar month, or the average of all composite samples taken in a given calendar month.

National Pollutant Discharge Elimination System (NPDES)
The national program for issuing, modifying, revoking and reissuing, terminating, monitoring and enforcing discharge permits from point sources to waters of the United States, and imposing and enforcing pretreatment requirements, under sections 307, 402, 318, and 405 of the CWA.

National Pretreatment Standard or Pretreatment Standard or Standard
[40 CFR §403.3(j)] Any regulation containing pollutant discharge limits promulgated by the EPA in accordance with section 307(b) and (c) of the Act, which applies to Industrial Users. This term includes prohibitive discharge limits established pursuant to §403.5.

New Source *[40 CFR §403.3(k)]*
Any building, structure, facility or installation from which there is or may be a discharge of pollutants, the construction of which commenced after the publication of proposed Pretreatment Standards under section 307(c) of the Act which will be applicable to such source if such standards are thereafter promulgated in accordance with that section *provided tha*t:
(a) The building, structure, facility or installation is constructed at a site at which no other discharge source is located; or
(b) The building, structure, facility or installation totally replaces the process or production equipment that causes the discharge of pollutants at an existing source; or
(c) The production or wastewater generating processes of the building, structure, facility, or installation are substantially independent of an existing source at the same site. In determining whether these are substantially independent, factors such as the extent to which the new facility is integrated with the existing plant, and the extent to which the new facility is engaged in the same general type of activity as the existing source, should be considered.
Construction on a site at which an existing source is located results in a modification rather than a new source if the construction does not create a new building, structure, facility, or installation meeting the criteria of paragraphs (k)(1)(ii), or (k)(1)(iii) of this section but otherwise alters, replaces, or adds to existing processor production equipment.
Construction of a new source, as defined under this paragraph has commenced if the owner or operator has:
(i) Begun, or caused to begin as part of a continuous onsite construction program:

(A) Any placement, assembly, or installation of facilities or equipment; or
(B) Significant site preparation work including clearing, excavation, or removal of existing buildings, structures, or facilities which is necessary for the placement, assembly, or installation of new source facilities or equipment, or
(C) Entered into a binding contractual obligation for the purchase of facilities or equipment which are intended to be used in its operation within a reasonable time. Options to purchase or contracts which can be terminated or modified without substantial loss, and contracts for feasibility, engineering, and design studies do not constitute a contractual obligation under this paragraph.

90-Day Final Compliance Report *[40 CFR §403.12(d)]*
A report submitted by categorical industrial users within 90 days following the date for final compliance with the standards. This report must contain flow measurement (of regulated process streams and other streams), measurement of pollutants, and a certification as to whether the categorical standards are being met.

Nonconventional Pollutants
Any pollutant that is neither a toxic pollutant nor a conventional pollutant (e.g., manganese, ammonia, etc.)

Non-Contact Cooling Water
Water used for cooling which does not come into direct contact with any raw material, intermediate product, waste product, or finished product. The only pollutant contributed from the discharge is heat.

Non-Regulated Wastestream
Unregulated and dilute wastestreams (not regulated by categorical standards).

Pass Through *[40 CFR §403.3(n)]*
A discharge which exits the POTW into waters of the United States in quantities or concentrations which, alone or in conjunction with a discharge or discharges from other sources, is a cause of a violation of any requirement of the POTW's NPDES permit (including an increase in the magnitude or duration of a violation).

Periodic Compliance Report *[paraphrased from 40 CFR §403.12(e) & (h)]*
A report on compliance status submitted by categorical industrial users and significant noncategorical industrial users to the control authority at least semiannually (once every six months).

Point Source *[40 CFR 122.2]*
Any discernible, confined, and discrete conveyance, including but not limited to any pipe, ditch, channel, tunnel, conduit, well, discrete fixture, container, rolling stock concentrated animal feeding operation vessel, or other floating craft from which pollutants are or may be discharged.

Pollutant *[40 CFR 122.2]*
Dredged spoil, solid waste, incinerator residue, filter backwash, sewage, garbage, sewage sludge, munitions, chemical wastes, biological materials, radioactive materials (except those regulated under the Atomic Energy Act of 1954, as amended (42 U.S.C. 2011 et seq.)), heat, wrecked or discarded equipment, rock, sand, cellar dirt, and industrial, municipal and agricultural waste discharged into water.

Pretreatment *[paraphrased from 40 CFR §403.3(q)]*
The reduction of the amount of pollutants, the elimination of pollutants, or the alteration of the nature of pollutant properties in wastewater prior to or in lieu of discharging or otherwise introducing such pollutants into a POTW.

Pretreatment Requirements *[40 CFR §403.3(r)]*
Any substantive or procedural requirement related to Pretreatment, other than a National Pretreatment Standard, imposed on an Industrial User.

Pretreatment Standards for Existing Sources (PSES)
Categorical Standards and requirements applicable to industrial sources that began construction prior to the publication of the proposed pretreatment standards for that industrial category. (see individual standards at 40 CFR Parts 405-471.)

Pretreatment Standards for New Sources (PSNS)
Categorical Standards and requirements applicable to industrial sources that began construction after the publication of the proposed pretreatment standards for that industrial category. (see individual standards at 40 CFR Parts 405-471.)

Priority Pollutant
Pollutant listed by the Administrator of the EPA under Clean Water Act section 307(a). The list of the current 126 Priority Pollutants can be found in 40 CFR Part 423 Appendix A.

Process Wastewater
Any water which, during manufacturing or processing, comes into contact with or results from the production or use of any raw material, intermediate product, finished product, byproduct, or waste product.

Production-Based Standards
A discharge standard expressed in terms of pollutant mass allowed in a discharge per unit of product manufactured.

Publicly Owned Treatment Works (POTW) *[40 CFR §403.3(o)]*
A treatment works as defined by section 212 of the Act, which is owned by a State or municipality (as defined by section 502(4) of the Act). This definition includes any devices or systems used in the storage, treatment, recycling, and reclamation of municipal sewage or industrial wastes of a liquid nature. It also includes sewers, pipes or other conveyances only if they convey wastewater to a POTW Treatment Plant.

The term also means the municipality as defined in section 502(4) of the Act, which has jurisdiction over the Indirect Discharges to and the discharges from such a treatment works.

Regulated Wastestream
For purposes of applying the combined wastestream formula, a wastestream from an industrial process that is regulated by a categorical standard.

Removal Credit *[paraphrased from 40 CFR §403.7]*
Variance from a pollutant limit specified in a categorical pretreatment standard to reflect removal by the POTW of said pollutant.

Representative Sample
A sample from a wastestream that is as nearly identical as possible in composition to that in the larger volume of wastewater being discharged and typical of the discharge from the facility on a normal operating day.

Sanitary Sewer Overflow (SSO)
Untreated or partially treated sewage overflows from a sanitary sewer collection system.

Self-Monitoring
Sampling and analyses performed by a facility to ensure compliance with a permit or other regulatory requirements.

Sewer Use Ordinance (SUO)
A legal mechanism implemented by a local government entity which sets out, among others, requirements for the discharge of pollutants into a publicly owned treatment works.

Significant Industrial User (SIU) *[paraphrased from 40 CFR §403.3(t)]*
(1) All users subject to Categorical Pretreatment Standards under 40 CFR 403.6 and 40 CFR chapter I, subchapter N; and (2) Any other industrial user that: discharges an average of 25,000 gallons per day or more of process wastewater to the POTW (excluding sanitary, noncontact cooling and boiler blowdown wastewater); contributes a process wastestream which makes up 5 percent or more of the average dry weather hydraulic or organic capacity of the POTW treatment plant; or is designated as such by the Control Authority as defined in 40 CFR 403.12(a) on the basis that the industrial user has a reasonable potential for adversely affecting the POTW's operation or for violating any pretreatment standard or requirement (in accordance with 40 CFR 403.8(f)(6)].

Significant Noncompliance (SNC) *[40 CFR §403.8(f)(2)(vii)]*
Industrial user violations meeting one or more of the following criteria:
1) Chronic violations of wastewater discharge limits, defined here as those in which sixty-six percent or more of all of the measurements taken during a six month period exceed (by any magnitude) the daily maximum limit or the average limit for the same pollutant parameter;
2) Technical Review Criteria (TRC) violations, defined here as those in which thirty-three percent or more of all of the measurements for each pollutants parameter taken during a six-month period equal or exceed the product of the daily maximum limit or the average limit multiplied by the applicable TRC (TRC=1.4 for BOD, TSS, fats, oil, and grease, and 1.2 for all other pollutants except pH);
3) Any other violation of a pretreatment effluent limit (daily maximum or longer-term average) that the Control Authority determines has caused, alone or in combination with other dischargers, interference or pass through (including endangering the health of POTW personnel or the general public);
4) Any discharge of a pollutant that has caused imminent endangerment to human health, welfare or to the environment or has resulted in the POTW's exercise of its emergency authority under paragraph (f)(1)(vi)(B) of this section to halt or prevent such a discharge;
5) Failure to meet, within 90 days after the schedule date, a compliance schedule milestone contained in a local control mechanism or enforcement order for starting construction, completing construction, or attaining final compliance;
6) Failure to provide, within 30 days after the due date, required reports such as baseline monitoring reports, 90-day compliance reports, periodic self-monitoring reports, and reports on compliance with compliance schedules;
7) Failure to accurately report noncompliance;
8) Any other violation or group of violations which the Control Authority determines will adversely affect the operation or implementation of the local pretreatment program.

Slug Discharge *[40 CFR §403.8(f)(2)(v)]*
Any discharge of a non-routine, episodic nature, including but not limited to, an accidental spill or a non-customary batch discharge.

Specific Prohibitions *[40 CFR §403.5(b)]*
The following pollutants shall not be introduced into a POTW:
1) Pollutants which create a fire or explosion hazard in the POTW, including but not limited to, wastestreams with a closed cup flashpoint of less than 140 degrees Fahrenheit or 60 degrees Centigrade using the test methods specified in 40 CFR Part 261.21;
2) Pollutants which will cause corrosive structural damage to the POTW, but in no case discharges with pH lower than 5.0, unless the works is specifically designed to accommodate such discharges;
3) Solid or viscous pollutants in amounts which will cause obstruction to the flow in the POTW resulting in interference;
4) Any pollutant, including oxygen-demanding pollutants (BOD, etc.) Released in a discharge at a flow rate and/or concentration which will cause interference with the POTW;
5) Heat in amounts which will inhibit biological activity in the POTW resulting in interference, but in no case heat in such quantities that the temperature at the POTW treatment plant exceeds 40°C (104°F) unless the Approval Authority, upon request of the POTW, approves alternative temperature limits;
6) Petroleum oil, nonbiodegradable cutting oil, or products of mineral oil origin in amounts that will cause interference or pass through;
7) Pollutants which result in the presence of toxic gases, vapors, or fumes within the POTW in a quantity that may cause acute worker health and safety problems;
8) Any trucked or hauled pollutants, except at discharge points designated by the POTW.

Standard Industrial Classification (SIC)
A system developed by the U.S. Office of Management and Budget that is used to classify various types of business entities. Effective in 1998, the SIC scheme is replace by the North American Industry Classification System (**NAICS**), although the EPA has not yet implemented this change.

Storm Water
Rain water, snowmelt, and surface runoff and drainage.

Time Proportional Composite Sample
A sample consisting of a series of aliquots collected from a representative point in the discharge stream at equal time intervals over the entire discharge period on the sampling day.

Toxic Pollutant
Any pollutant listed as toxic under section 307(a)(1) of the CWA, or in the case of sludge use or disposal practices, any pollutant identified in regulations implementing section 405(d) of the CWA.

Toxicity Reduction Evaluation
A site-specific study conducted in a stepwise process designed to identify the causative agent(s) of effluent toxicity, isolate the sources of toxicity, evaluate the effectiveness of toxicity control options, and then confirm the reduction in effluent toxicity.

Toxicity Test
A procedure to determine the toxicity of a chemical or an effluent using living organisms. A toxicity test measures the degree of effect on exposed test organisms of a specific chemical or effluent.

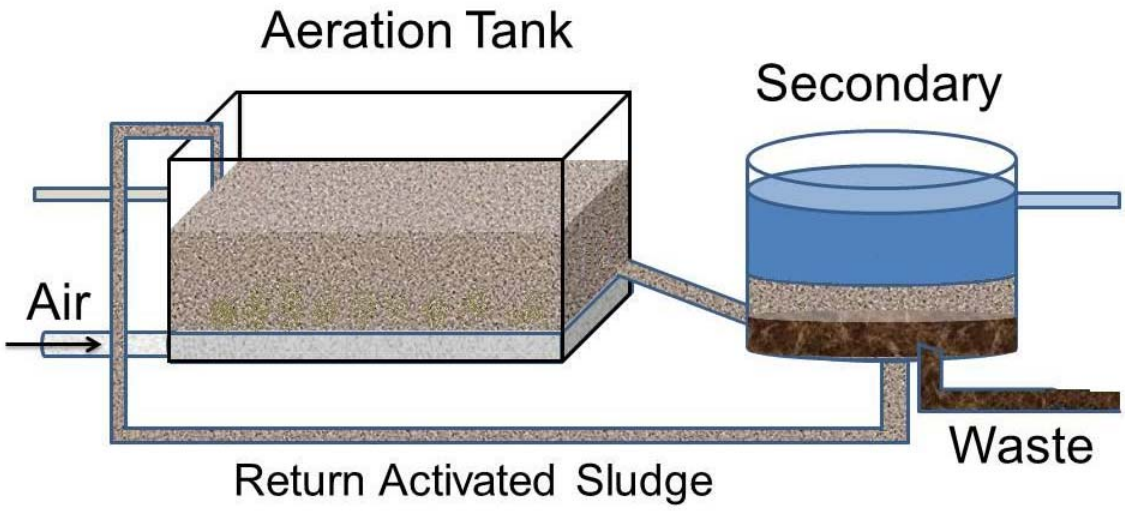
Toxicity Identification Evaluation
Set of procedures to identify the specific chemicals responsible for effluent toxicity.

Unregulated Wastestream
For purposes of applying the combined wastestream formula, a wastestream not regulated by a categorical standard nor considered a dilute wastestream.

Upset *[paraphrased from 40 CFR §403.16(a)]*
An exceptional incident in which there is unintentional and temporary noncompliance with categorical Pretreatment Standards because of factors beyond the reasonable control of the Industrial User. An Upset does not include noncompliance to the extent caused by operational error, improperly designed treatment facilities, inadequate treatment facilities, lack of preventative maintenance, or careless or improper operation.

Water Quality Criteria
Comprised of both numeric and narrative criteria. Numeric criteria are scientifically derived ambient concentrations developed by EPA or States for various pollutants of concern to protect human health and aquatic life. Narrative criteria are statements that describe the desired water quality goal.

Water Quality Standard
A statute or regulation that consists of the beneficial designated use or uses of a waterbody, the numeric and narrative water quality criteria that are necessary to protect the use or uses of that particular waterbody, and an antidegradation statement.



Wastewater Sampling Information

Required Containers, Preservation Techniques, and Holding Times
40 CFR 136.3

Parameter No./name Container Preservation Maximum holding time

Table IA--Bacteria Tests:
1-4 Coliform, fecal and total. P,G..... Cool, 4C, 0.008% $Na_2S_2O_3$..... 6 hours.
5 Fecal streptococci..... P,G..... Cool, 4C, 0.008% $Na_2S_2O_3$ ..... 6 hours.
Table IA--Aquatic Toxicity Tests:
6-10 Toxicity, acute and chronic. P,G..... Cool, 4C ..... 36 hours.

Table IB--Inorganic Tests:
1. Acidity..... P, G..... Cool, 4C..... 14 days.
2. Alkalinity..... P, G..... Cool, 4C..... 14 days.
4. Ammonia..... P, G..... Cool, 4C, H_2SO_4 to pH< 2..... 28 days.
9. Biochemical oxygen demand.. P, G..... Cool, 4C..... 48 hours.
10. Boron..... P, PFTE, or HNO_3 TO pH2..... 6 months. Quartz.

11. Bromide..... P, G..... None required..... 28 days.
14. Biochemical oxygen demand, P, G..... Cool, 4C..... 48 hours. carbonaceous.
15. Chemical oxygen demand.... P, G..... Cool, 4C, H_2SO_4 to pH<2..... 28 days.
16. Chloride..... P, G..... None required..... 28 days.
17. Chlorine, total residual.. P, G..... None required ..... Analyze immediately.
21. Color..... P, G..... Cool, 4C..... 48 hours.
23-24. Cyanide, total and amenable to chlorination. P, G..... Cool, 4 deg.C, NaOH to pH>12, 0.6g Ascorbic acid 14 days.
25. Fluoride..... P..... None required..... 28 days.
27. Hardness..... P, G..... HNO_3 to pH<2, H_2SO_4 to pH <2..... 6 months.
28. Hydrogen ion (pH)..... P, G..... None required..... Analyze immediately.
31, 43. Kjeldahl and organic nitrogen. P, G..... Cool, 4 deg.C, H_2SO_4 to pH <2..... 28 days.

Metals:
18. Chromium VI..... P, G..... Cool, 4C..... 24 hours.
35. Mercury..... P, G..... HNO_3 to pH<2..... 28 days.
3, 5-8, 12, 13, 19, 20, 22, P, G..... .....do..... 6 months.
26, 29, 30, 32-34, 36, 37, 45, 47, 51, 52, 58-60, 62,63, 70-72, 74, 75. Metals, except boron, chromium VI and mercury.

38. Nitrate..... P, G..... Cool, 4 deg.C..... 48 hours.
39. Nitrate-nitrite..... P, G..... Cool, 4 deg.C, H_2SO_4 to pH <2..... 28 days.
40. Nitrite..... P, G..... Cool, 4 deg.C..... 48 hours.
41. Oil and grease..... G..... Cool to 4 deg.C, HCl or H_2SO_4 to pH <2 to 28 days.
42. Organic Carbon..... P, G..... Cool to 4 deg.C HC1 or H_2SO_4 to pH <2 or 28 days.
44. Orthophosphate..... P, G..... Filter immediately, Cool, 4 deg.C. ..... 48 hours.
46. Oxygen, Dissolved Probe... G Bottle and top. None required..... Analyze immediately.
47. Winkler..... ..... G Bottle and top. Fix on site and store in dark... 8 hours.
48. Phenols..... G only..... Cool, 4 deg.C,..... H_2SO_4 to pH <2 28 days.
49. Phosphorus (elemental).... G..... Cool, 4 deg.C..... 48 hours.
50. Phosphorus, total..... P, G..... Cool, 4 deg.C, H_2SO_4 to pH <2……..28 days.
53. Residue, total..... P, G..... Cool, 4 deg.C..... 7 days.
54. Residue, Filterable..... P, G..... Cool, 4 deg.C..... 7 days.
55. Residue, Nonfilterable P, G..... Cool, 4 deg.C..... 7 days.

56. Residue, Settleable (TSS)..... P, G..... Cool, 4 deg. C..... 48 hours.
57. Residue, volatile..... P, G..... Cool, 4 deg. C..... 7 days.
61. Silica..... P, PFTE, or Quartz……. Cool, 4 deg. C..... 28 days.
64. Specific conductance..... P, G..... . Cool, 4 deg. C..... 28 days.
65. Sulfate..... P, G..... ..... Cool, 4 deg. C ..... 28 days.
66. Sulfide.... P, G..... Cool, 4 deg. C add zinc acetate plus sodium hydroxide to pH>9. 7 days.
67. Sulfite..... P, G..... None required..... Analyze immediately.
68. Surfactants..... P ,G..... Cool, 4 deg. C..... 48 hours.
69. Temperature..... P, G..... None required..... Analyze.
73. Turbidity..... P, G..... Cool, 4 deg. C..... 48 hours.

Table IC--Organic Tests
13, 18-20, 22, 24-28, 34-37, G, Teflon-lined septum Cool, 4 deg. C, 0.008% $NA_2S_2O_3$ 14 days.
39-43, 45-47, 56, 76, 104, 105, 108-111, 113.
Purgeable Halocarbons. 6, 57, 106.
Purgeable aromatic hydrocarbons G, Teflon-lined septum Cool, 4 deg.C, 0.008% $NA_2S_2O_3$ 14 days.
3, 4. Acrolein and acrylonitrile G, Teflon-lined septum Cool, 4 deg.C, 0.008% $NA_2S_2O_3$ pH 4-5 14 days.
23, 30, 44, 49, 53, 77, 80, 81, 98, 100, 112. G, Teflon-lined Cool, 4 deg.C, 0.008% $NA_2S_2O_3$ 14 days.
Phenols G, Teflon-lined septum Cool, 4 deg.C, 0.008% $NA_2S_2O_3$ pH 4-5 7 days until extraction; 40 days after extraction.
7, 38. Benzidines G, Teflon-lined septum…… Cool, 4 deg.C, 0.008% $NA_2S_2O_3$ 7 days until extraction.
14, 17, 48, 50-52. Phthalate G, Teflon-lined septum Cool, 4 deg.C..... 7 days until extraction; esters 40 days after extraction.
82-84. Nitrosamines G, Teflon-lined septum ……Cool, 4 deg. C, 0.008% $NA_2S_2O_3$ Store in dark
88-94. PCBs G, Teflon-lined septum Cool, 4 deg.C ..... 7 days until extraction; 40 days after extraction.
54, 55, 75, 79. Nitroaromatics ..... G, Teflon-lined septum…….Cool, 4 deg.C, 0.008% $NA_2S_2O_3$ and isophorone
1, 2, 5, 8-12, 32, 33, 58, 59, 74, 78, 99, 101. Polynuclear aromatic hydrocarbons. Cool, 4 deg.C, 0.008% $NA_2S_2O_3$ Store in dark
15, 16, 21, 31, 87. Haloethers ..... G, Teflon-lined septum..... Cool, 4 deg.C, 0.008% $NA_2S_2O_3$ 7 days until extraction; 40 days after extraction.
29, 35-37, 63-65, 73, 107. Chlorinated hydrocarbons G, Teflon-lined septum…….Cool, 4 deg.C, 7 days until extraction; 40 days after extraction.
60-62, 66-72, 85, 86, 95-97, 102, 103. CDDs/CDFs aqueous: field and lab G..... Cool, 0-4 deg.C, pH9, 0.008% $NA_2S_2O_3$ 1 year preservation.
Solids, mixed phase, and .....do..... Cool, 4 deg.C..... 7 days. Tissue: field preservation.
Solids, mixed phase, and .....do..... Freeze, -10 deg.C..... 1 year. Tissue: lab preservation.

Table ID--Pesticides Tests:
1-70. Pesticides \11\..... .....do..... Cool, 4 deg.C, pH 5-9 ..... Do.
Table IE--Radiological Tests:
1-5. Alpha, beta and radium... P, G..... HNO_3 to pH2..... 6 months.

Polyethylene (P) or glass (G). For microbiology, plastic sample containers must be made of sterilizable materials (polypropylene or other autoclavable plastic).

Sample preservation should be performed immediately upon sample collection. For composite chemical samples each aliquot should be preserved at the time of collection. When use of an automated sampler makes it impossible to preserve each aliquot, then chemical samples may be preserved by maintaining at 4 degrees C until compositing and sample splitting is completed.

When any sample is to be shipped by common carrier or sent through the United States Mails, it must comply with the Department of Transportation Hazardous Materials Regulations (49 CFR part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirements of Table II, the Office of Hazardous Materials, Materials Transportation Bureau, Department of Transportation has determined that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater); Nitric acid (HNO_3in water solutions at concentrations of 0.15% by weight or less (pH about 1.62 or greater); Sulfuric acid (H_2SO_4) in water solutions at concentrations of 0.35% by weight or less (pH about 1.15 or greater); and Sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before analysis and still be considered valid. Samples may be held for longer periods only if the permittee, or monitoring laboratory, has data on file to show that for the specific types of samples under study, the analytes are stable for the longer time, and has received a variance from the Regional Administrator under Sec. 136.**3**(e). Some samples may not be stable for the maximum time period given in the table. A permittee, or monitoring laboratory, is obligated to hold the sample for a shorter time if knowledge exists to show that this is necessary to maintain sample stability. See Sec. 136.**3**(e) for details. The term ``analyze immediately'' usually means within 15 minutes or less of sample collection. Should only be used in the presence of residual chlorine.

Maximum holding time is 24 hours when sulfide is present. Optionally all samples may be tested with lead acetate paper before pH adjustments in order to determine if sulfide is present. If sulfide is present, it can be removed by the addition of cadmium nitrate powder until a negative spot test is obtained. The sample is filtered and then NaOH is added to pH 12.

Samples should be filtered immediately on-site before adding preservative for dissolved metals.

Guidance applies to samples to be analyzed by GC, LC, or GC/MS for specific compounds.

Sample receiving no pH adjustment must be analyzed within seven days of sampling.

The pH adjustment is not required if acrolein will not be measured. Samples for acrolein receiving no pH adjustment must be analyzed within **3** days of sampling.

When the extractable analytes of concern fall within a single chemical category, the specified preservative and maximum holding times should be observed for optimum safeguard of sample integrity. When the analytes of concern fall within two or more chemical categories, the sample may be preserved by cooling to 4 deg. C, reducing residual chlorine with 0.008% sodium thiosulfate, storing in the dark, and adjusting the pH to 6-9; Samples preserved in this manner may be held for seven days before extraction and for forty days after extraction. Exceptions to this optional preservation and holding time procedure are noted in footnote 5 (re the requirement for thiosulfate reduction of residual chlorine), and footnotes 12, 13 (re the analysis of benzidine).

If 1,2-diphenylhydrazine is likely to be present, adjust the pH of the sample to 4.0<plus-minus>0.2 to prevent rearrangement to benzidine.

Extracts may be stored up to 7 days before analysis if storage is conducted under an inert (oxidant-free) atmosphere.

For the analysis of diphenylnitrosamine, add 0.008% $NA_2S_2O_3$ and adjust pH to 7-10 with NaOH within 24 hours of sampling.

The pH adjustment may be performed upon receipt at the laboratory and may be omitted if the samples are extracted within 72 hours of collection. For the analysis of aldrin, add 0.008% $NA_2S_2O_3$.

Sufficient ice should be placed with the samples in the shipping container to ensure that ice is still present when the samples arrive at the laboratory. However, even if ice is present when the samples arrive, it is necessary to immediately measure the temperature of the samples and confirm that the $4^{O}C$ temperature maximum has not been exceeded.

In the isolated cases where it can be documented that this holding temperature cannot be met, the permittee can be given the option of on-site testing or can request a variance. The request for a variance should include supportive data which show that the toxicity of the effluent samples is not reduced because of the increased holding temperature.

Common Wastewater Sample Collection Bottles

Above, 625/608, 1657, TTO/Organics, TPH/Oil/Grease
Smaller bottles-TOCs, VOCs, 601/602 and 502.2.

Wastewater/Pretreatment Sampling General Information

In accordance with the Clean Water Act and General Pretreatment Program Regulations, the POTW conducts a variety of sampling activities which must be closely coordinated.

Each of these activities is briefly described below.

Permit Application Policy Example
All industrial users that require a permit must be sampled to determine the characteristics of the wastes to be discharged into the POTW's sewer system. Prior to the issuance of a permit for existing industrial users, the POTW samples the user's effluent, and performs the analyses required by the applicable discharge standards (i.e., Categorical standards or local limits).

For new industrial users, estimates of the wastes to be discharged into the POTW's sewer system must be submitted along with the permit application. No sampling would be performed at these new facilities, since they do not presently discharge wastes into the sewer system. A four-day sampling program is usually conducted at each site to collect both composite and grab (for pollutants not amenable to composite sampling) samples as needed.

Sewer System Evaluation Policy Example
On a regular basis, selected locations in the sewer system are sampled to develop background data for purposes of updating the local limits, and to screen areas for higher than ***"background"*** pollutant levels. In addition, problem areas are sampled on an as needed basis to determine potential sources of POTW Code violations that either occur on a frequent basis, or are the result of a slug load to the sewer system. To monitor sewers for background information, the sampling program would typically be conducted over a four-day period. In instances where the intent is to determine sources of pollutants and/or slug loads, the length of the program would vary.

Multi-City Users (Metering Stations) Policy Example
All wastewater, which is transported to the POTW Treatment Plant from the Multi-City users, is analyzed for pollutants of concern to the Industrial Pretreatment Program. The sampling program is conducted over a five-day period to obtain four days of sampling data at each sewer location (i.e., a metering station) on a quarterly basis. Once the sampling dates have been determined, the Water Quality Inspector will notify, in writing, the Sub-regional Organizational Group (**SROG**) or equivalent representative for that City of the dates when the sampling will be conducted.

Sampling Safety Policy Example
Upon arrival at the site, safety is the priority. A visual inspection must be completed prior to any entry. The site must be free of any obstructions or hazards which may cause injury when entering the sampling area. If there are any problems detected, the SROG or equivalent representative and the Water Quality Inspector should be notified, and no entry should be attempted until the problem has been corrected.

Metering and Sampling Stations Qualify As Confined Spaces
If all safety criteria have been met, prepare equipment for the site. Check the assignment sheet to determine what parameters are required to be sampled, which in turn determines the type of tubing to be used, (i.e. Tygon or Teflon).

The sampler must be completely assembled before performing QA/QC procedures. After QA/QC is complete, a sufficient amount of weight must be attached to the tubing to keep the strainer submerged in the effluent for proper siphoning of the sample, without allowing the strainer to hit the bottom of the flume. Make sure the intake tubing does not kink.

If the metering station has a flow meter, you may connect either their cable or a POTW cable to the sampler from the flow meter. Occasionally, you will set up a flow meter to have a comparison reading. Determine the pulse rate and proper setting from the flow, and program the sampler. After entering the data into the sampler, wait to make sure the equipment is pulling samples. After the initial set-up of the sampling equipment, samples will be collected during the remainder of the sampling period. Split samples may be requested by the SROG or equivalent representative. If the volume of the sample is adequate, these may be given, provided the representative supplies the containers and allows the POTW Inspector to pour off the samples.

Upon exiting the confined space, continue to follow the confined space entry procedures as outlined by OSHA Standards. When you return to the sampling vehicle, you must immediately perform field tests and preserve the samples according to the techniques set forth by Standard Methods or the State/Federal Rule.

All paper work must be filled out completely before the sampling crew's departure. This paperwork includes the chain of custody which is turned in to the laboratory with the samples, "**Metering Station Field Observation Form**" or equivalent form that remains with the sampling site file, and the Multi-City Metering Station Sample Record of which the original is given to the Water Quality Inspector or representative and the copy is given to the SROG or equivalent representative. If there is not a representative at the site, these copies will be turned over to the Water Quality Inspector with the originals at the end of the week.

Remember, all paperwork should be completed prior to leaving site or sampling location.

Wastewater and Pretreatment Compliance Monitoring

There are two types of sampling activities that are performed as part of compliance monitoring for permitted industries: unscheduled and demand.

Unscheduled sampling is used to determine the compliance status of the user. Instances of noncompliance are often identified during unannounced monitoring visits. No notice is given for this type of sampling. This type of sampling is performed two to four times a year, at each industrial user site, over a two to five-day period to obtain sampling data

Demand sampling is usually initiated in response to a known or suspected violation, discovered as a result of a self-monitoring report, routine sampling visit, public complaint, unusual influent condition at the wastewater treatment plant, or emergency situations (e.g., plant upsets, sewer line blockages, fires, explosions, etc.).

Most often, this type of sampling is conducted to support enforcement actions against an industrial user. This type of sampling activity is performed on an as needed basis. The length of the sampling program depends on the flow, nature of the wastes, and type of samples (i.e., grab or composite) to be collected. Typically, composite and grab samples are collected at each user site.

Nonpermitted Industrial Users (User Rate Charge Program) Policy Example
On a periodic basis (i.e., once every two to three years), commercial and minor industrial users are sampled to determine discharge concentrations of various pollutants. Typical types of users which may be sampled include: restaurants, photo processing laboratories, laundries, car washes, and printing shops.

A three- to four-day sampling program is usually conducted at each assigned site. Commercial establishments are sampled to establish BOD and SS levels for various groups of users for the POTW's Finance or Utilities Division.

This activity is also helpful in identifying industrial or commercial users which may discharge pollutants of concern.

Wastewater Treatment Plant Sampling
POTW samples are collected in accordance with the National Pollutant Discharge Elimination System (**NPDES**) permit which sets discharge limits for certain pollutants and specifies sampling frequencies and sample types.

The POTW is responsible for coordinating the plant sampling activity with laboratory personnel who prepare any special sampling bottles and laboratory appurtenances necessary (i.e. trip blanks, etc.) to complete the sampling objectives.

Pre-Treatment Monitoring Locations Should:

- be appropriate for waste stream conditions;
- be representative of the discharge;
- have no bypass capabilities; and
- allow for unrestricted access at all times.

Control Authorities should measure flow to allow for collection of flow-proportioned composite samples, which are required, unless flow-proportional sampling is not feasible. Flow-proportional composite samples are preferred over time composite samples particularly where the monitored discharge is intermittent or variable.

Desired analyses dictate the preparation protocols, equipment, and collection bottles to use to avoid contamination of samples or loss of pollutants through improper collection. Sampling for such pollutants as pH, cyanide, oil and grease, flashpoint, and volatile organic compounds require manual collection of grab samples. Similar to composite samples, grab samples must be representative of the monitored discharge and are to be collected from actively flowing wastestreams. Fluctuations in flow or the nature of the discharge may require collection of and hand-compositing of more than one grab sample to accurately access compliance.

To ensure defensibility of data, Control Authorities should develop and implement standard operating procedures and policies detailing sample collection and handling protocols in accordance with 40 CFR Part 136. Adherence to proper sample collection and handling protocols, 40 CFR Part 136 approved analytical methodologies, and record keeping requirements [40 CFR §403.12(o)(1)] can be verified through review of field measurement records, chain of custodies, and lab reports. Field measurement records may require information regarding sample location, condition of and programmed settings for sampling equipment, wastewater meter readings, and information for such parameters as pH and temperature which require analysis in the field.

Chain of custody forms serve as a link between field personnel and the laboratory and contain information regarding the sample matrix, type, and handling. Lab reports should contain the minimum information specified in 40 CFR §403.12(o)(1)(ii-iv) as well as any additional information necessary to demonstrate compliance with 40 CFR Part 136 requirements (e.g., analytical methodology, sample preparation date and time, time of analysis).

Use of standardized forms which prompt recording of information necessary for demonstrating compliance with applicable requirements, will aid in ensuring it can be used as admissible evidence in enforcement proceedings or in judicial actions.

Wastewater Plant Sampling Procedure Example

Set up two samplers or equivalent at the plant influent channel and two samplers at the plant effluent channel. Two samplers are used to provide sufficient sample quantity and to minimize sampler failure. All sampling equipment must be prepared and cleaned as established in your POTW's procedures. Teflon hose or equivalent is required. Sampling sites are specified in each plants NPDES permit.

Collect the following composite samples at all sites.

(1) Metals Sample - (one 2-liter plastic bottle)

Preserve with 1:1 nitric acid to a pH < 2. Store sample on ice to four degrees Centigrade.

(2) Cyanide Sample - one (2-liter plastic bottle)

Collect the cyanide sample as a composite in accordance with NPDES permit. Check the sample for chlorine. If Cl_2 is present, use ascorbic acid to eliminate chlorine. Add NaOH to a pH > 12. Store samples on ice to four degrees Centigrade.

(3) EPA Test Method 608 and 625[i] samples are informational samples only. These results are used for local limits data.

608 and 625 samples are collected as composite samples. At the influent channel: Collect one 1-liter amber glass bottle of each sample (608, 625). Check samples for chlorine. At the effluent channel: Collect one 4-liter amber glass bottle of each sample (608, 625). Check samples for chlorine. If Cl_2 is present in the samples, use sodium thiosulfate ($Na_2S_20_3$) to eliminate chlorine. Store samples on ice to four degrees Centigrade.

(4) **625/Phenols** are collected as a grab sample. Collect one 4-liter amber glass bottle at the effluent channel only. Check the sample for chlorine. If Cl_2 is present, use sodium thiosulfate ($Na_2S_20_3$) to eliminate chlorine. Store sample on ice to four degrees Centigrade.

Bio-Solids Sampling Example

Bio-solids (dried sludge) samples are collected at POTWs. Normally, bio-solid samples will be collected from the final storage area for dry sludge. The location of the dried bio-solids may vary based on the individual plants. Sampling frequency will be determined on an as needed basis and to comply with the EPA requirements.

All samples collected are grabs. All samples are collected using a sterile plastic scoop or equivalent in order to avoid any contamination.

The following is a list of samples that are normally collected:

PARAMETER	CONTAINER
Helminth Ova & Enteric Virus	1 Qt Plastic Bag (Ziploc)
Metals +	500 ml Plastic Bottle
Nitrogen (total)	4 oz Glass Bottle
TOC (Total Organic Carbon)	4 oz Glass Bottle
Fecal Coliform	(autoclaved from lab)
6 hr hold time	500 ml Plastic Bottle

Sample Scheduling Example
An active file is maintained on each sampling location which contains historical data, including past process discharge flow readings, water meter readings, sampling dates, and conditions of sampling site.

Treated Wastewater Effluent River Sampling Activities Example
When developing a sampling plan for river sampling, the following considerations must be observed:

(1) Sampling sites must meet the objectives of the program or study.
(2) At the sampling sites the river must be flowing freely and the sample must be as representative as possible of river flow at that site. Consideration of all safety factors must be observed.
(3) Samples must be collected at midstream of the main channel at approximately two-thirds of the depth unless specific depths have been requested.
(4) All safety precautions must be observed during sampling which includes the use of harnesses, waterproof boots and other equipment.

Samples from Sewers *Example*
Sewer system and user rate sampling are conducted in manholes. General guidelines for selection of sampling locations include the following:

(1) Samples should be taken at points of high turbulent flow to ensure good mixing and prevent the deposition of solids.
(2) The sample location should be easily accessible and free of any major safety hazards.
(3) Sample lines should not be located where there is surface scum.
(4) If a flow study or a flow/proportional sampling event is required, make sure that the sewer pipe does not have a curve, a drop in the line or any obstructions. These would cause false readings.

Co-Removal of Emerging Contaminants

This section provides a brief background on emerging contaminants and key findings from studies on the co-removal of emerging contaminants by nutrient removal technologies.

Background on Emerging Contaminants

The term "emerging contaminants" refers broadly to those synthetic or naturally occurring chemicals, or to any microbiological organisms, that have not been commonly monitored in the environment but which are of increasing concern because of their known or suspected adverse ecological or human health effects. Emerging contaminants can fall into a wide range of groups defined by their effects, uses, or by their key chemical or microbiological characteristics. Two groups of emerging contaminants that are of particular interest and concern at present are endocrine disrupting chemicals (EDCs) and pharmaceutical and personal care products (PPCPs). These compounds are found in the environment, often as a result of human activities.

EDCs may interfere with the endocrine systems by damaging hormone-producing tissues, changing the processes by which hormones are made or metabolized, or mimicking hormones. In addition to natural and synthetic forms of human hormones that are released into the environment, there are a multitude of synthetic organic compounds that are able to disrupt the endocrine system. Public concern about EDCs in the environment has been rapidly increasing since the 1990s when researchers reported unusual sexual characteristics in wildlife. A report by the USGS, found that fish in many streams had atypical ratios of male and female sex hormones (Goodbred et al., 1997). In England, researchers found that male trout kept in cages near WWTP outfalls were developing eggs on their testes and had increased levels of the protein that is responsible for egg production (vitellogenin) (Sumpter, 1995; Kaiser, 1996). Follow-up laboratory studies showed that synthetic forms of estrogen (17α-ethynylestradiol (EE2)) could increase vitellogenin production in fish at levels as low as 1-10 ng/L, with positive responses seen down to the 0.1-0.5 ng/L level (Purdom et al., 1994).

Human estrogens have the ability to alter sexual characteristics of aquatic species at trace concentrations as low as 1 ng/L (Purdom et al., 1994). WWTP effluents have been identified as a primary source for EDCs in the environment, with the bulk of their endocrine disrupting activity resulting from human estrogen compounds (Desbrow et al., 1998, Snyder et al., 2001). The synthetic estrogen, EE2, and the natural estrogens, estrone (E1) and 17β-estradiol (E2), are the greatest contributors to endocrine disrupting activity in WWTP effluent (Johnson et al., 2001) with EE2 showing the greatest recalcitrance in WWTPs (Joss et al., 2004). Influent concentrations range from below detection to 70 ng/L for EE2, 670 ng/L for E1 and 150 ng/L for E2 (Vethaak et al., 2005, Clara et al., 2005b). Other EDCs include tributyl tin, which was previously used in paints to prevent marine organisms from sticking to ships, nonylphenol (a surfactant), and bisphenol A (platicizer and preservative).

PPCPs encompass a wide variety of products that are used by individuals for personal health or cosmetic reasons, and also include certain agricultural and veterinary medicine products. PPCPs comprise a diverse collection of thousands of chemical substances, including prescription and over-the counter therapeutic drugs, veterinary drugs, fragrances, sun-screen products, vitamins, and cosmetics. Many of these products, notably the pharmaceuticals for human or animal use, are specifically designed to be biologically active, and some PPCPs may also fall into the category of EDCs described previously.

Estrogens of Concern

Name	***Chemical Structure***	***Name***	***Chemical Structure***
E1	Estrone		$C_{18}H_{22}O_2$
E2	17β-estradiol		$C_{18}H_{24}O_2$
E3	Estriol		$C_{18}H_{24}O_3$
EE2	17α-ethynylestradiol		$C_{20}H_{24}O_2$

Currently, municipal sewage treatment plants are engineered to remove conventional pollutants such as solids and biodegradable organic material but are not specifically designed for PPCP removal or for other unregulated contaminants. Wastewater treatment commonly consists of primary settling followed by biological treatment, secondary settling, and disinfection. This treatment can remove more than 90 percent of many of the most commonly known or suspected EDCs found in wastewater influent; however, low concentrations of some suspected EDCs may remain in the wastewater treatment sludge or effluent (WERF, 2005). As discussed in the next section, studies have shown enhanced nutrient removal technologies to be effective in removing low concentrations of some emerging contaminants.

Removal of Emerging Contaminants by Nutrient Removal Technologies
Several studies have examined the effectiveness of current wastewater treatment technologies in the removal of emerging contaminants. Some of these studies are discussed below and their major findings are organized under three subsections: role of activated sludge SRT in removal efficiency, role of nitrifying bacteria in biodegradation, and use of RO to improve removal efficiencies. Details regarding the study design, such as evaluated treatments and contaminants, and a summary of major study findings are provided at the end of this section.

The significant findings are also presented as follows:
• Removal efficiencies were enhanced for several investigated contaminants at longer SRTs, with critical SRTs for some beyond which removal rates did not improve.
• Longer SRTs allow for the establishment of slower growing bacteria (e.g., nitrifying bacteria in activated sludge), which in turn provide a more diverse community of microorganisms with broader physiological capabilities.
• Nitrifying bacteria may play a key role in biodegradation but the role of heterotrophic bacteria may also play a significant role.
• Reverse osmosis has been found to effectively remove PPCPs below detection limits including those that that were not consistently removed at longer SRTs.

One caveat regarding studies on emerging contaminants is that their concentrations in wastewater influent are often quite low (e.g., concentrations of ng/L to µg/L range) and may be close to method detection limits. Therefore, small variations between measured influent and effluent concentrations may show large variations in apparent removal efficiencies, possibly even producing negative calculated removals.

Role of Solids Retention Time in Removal Efficiency
The focus of several studies has been the relationship of the SRT to the removal of emerging contaminants. In particular, many investigated whether longer SRTs would result in increased removal efficiencies for estrogens and other categories of PPCPs. Longer activated sludge SRTs allow for the establishment of slower growing bacteria (e.g., nitrifying bacteria in activated sludge), which in turn provide a more diverse community of microorganisms with broader physiological capabilities.

Clara et al. (2005a), Kreuzinger et al. (2004), and Oppenheimer et al. (2007) observed enhanced removal with increasing SRTs for most of the EDCs and pharmaceuticals tested and found no significant differences in removal performances between conventional activated sludge systems and MBR when operated at similar SRT10 °C. This is likely due to the molecular weight of the study compounds, which was smaller than the molecular weight cut-off of the ultrafiltration membranes in the MBR.

Researchers have observed similar findings for natural estrogens with higher removal percentages at longer SRTs. Effluent concentrations for three natural estrogens were measured near their detection limits at SRTs10° C higher than 10 days, with their critical SRTs10° C estimated between 5 and 10 days (Clara et al., 2005a).

High removal rates of > 90 percent were also observed by Joss et al. (2004)in a study in which they evaluated the removal of E1, E2, and EE2 under aerobic and anaerobic conditions in WWTPs designed for nutrient removal. Joss et al. (2004) also reported that the maximum efficiency is dependent on redox conditions, with the highest removal rate occurring during the reduction of E1 to E2 under aerobic conditions. Clara et al. (2005a) cited examples where conflicting results were obtained for EE2.

Ternes et al. (1999) found no significant elimination of this compound during batch experiments; however, Baronti et al. (2000) and Joss et al. (2004) report greater than 85 percent removal in full-scale WWTPs. For the pharmaceuticals ibuprofen and bezafibrate, Clara et al. (2005a) reported more than 95 percent removal during treatment and calculated the critical value for SRT10° C at 5 days for ibuprofen and about 10 days for bezafibrate. Analogous removal results were obtained in several other studies (Stumpf et al., 1998; Buser et al., 1999; Zwiener et al., 2001, as cited in Clara et al., 2005a; Oppenheimer et al., 2007). Clara et al. (2005b) noted no or slight removal of these two pharmaceuticals and two musk fragrances (tonalide and galaxolide) at a WWTP with a low SRT of 1 to 2 days.

Clara et al. (2005a, 2005b) also found that the pharmaceutical carbamazepine was not removed during wastewater treatment. In addition, these studies found contradictory results for diclofenac (e.g., removal rates ranged from no removal to > 70 percent at SRTs of > 10 days (Clara et al., 2005b)). Clara et al. (2005a) also cited several examples where conflicting results were obtained for diclofenac. No significant removal was reported by Buser et al. (1999) and Heberer (2002a); whereas, Ternes et al. (1998) observed elimination rates of up to 70 percent.

Clara et al. (2005a, 2005b) concluded that the removal potential for conventional WWTPs and MBRs depends on the SRT. They further concluded that high removal rates can be achieved at SRTs10° C of more than 10 days. These parameters correspond to the design criteria for nitrogen removal in the German Association for Water, Wastewater and Waste (ATV-DVWK, 2000) and the urban wastewater directive of the European Community (91/271/EEC) for WWTPs in sensitive areas. In its 2005, technical brief, “Endocrine Disrupting Compounds and Implications for Wastewater Treatment,” WERF summarized information from several studies that examined the effectiveness of current wastewater treatment technologies in the removal of EDCs.

The classes of EDCs included:
steroids/sterols (naturally occurring, synthetic, and phytoestrogens), organohalides, metals/ organometals, alkyl phenols, polycyclic aromatic hydrocarbons (PAHs)/crude oil, and plasticizers.

Although the WERF 2005 technical brief states that in general, EDC treatment effectiveness is improved with increased SRT, it does not provide the specific SRTs that are associated with the cited removal rates.

Oppenheimer et al. (2007) examined the relationship of SRT to treatment removal efficiencies for 20 PPCPs that are commonly found in the influent of U.S. treatment facilities. Many of the studies already discussed here have been conducted primarily in Europe, were conducted at small-scale WWTPs and bench/pilot plants under controlled conditions, and focused on estrogens and prescription pharmaceuticals rather than PPCPs. The Oppenheimer et al. (2007) study also noted trends regarding the effect of HRT and pure oxygen systems compared to conventional aeration systems on PPCP removal.

Oppenheimer et al. (2007) defined a minimum critical SRT as the minimum time needed to consistently demonstrate greater than 80 percent removal. The results of the study showed that this critical SRT was compound dependent but that the majority of the 20 PPCPs were consistently removed in those treatment plants operating at SRTs of 5 to 15 days. Specifically, 9 of 12 frequently occurring PPCPs were effectively removed through secondary treatment (e.g., ibuprofen).

Conversely, six compounds that are routinely detected in influent (i.e., detected in at least 20 percent of the influent samples) were not well removed by secondary treatment (BHA, DEET, musk ketone, triclosan, benzophenone, galaxolide).

The results for galaxolide conflicted with those reported by Clara et al. (2005b) who generally found high removal rates with SRTs > 10 days and Kreuzinger et al. (2004) who reported removal at SRT between 25 to 40 days. Oppenheimer et al. (2007) found that some compounds such as octylphenol, tri-(chloroethyl) phosphate, and triphenylphosphate were not well removed by secondary treatment; however, these were seldom detected in the influent samples. Based on these results, Oppenheimer et al. (2007) concluded that secondary treatment provides an "effective first barrier" for the 20 PPCPs in the study.

Oppenheimer et al. (2007) also noted trends regarding the effect of HRT and pure oxygen systems compared to conventional aeration systems on PPCP removal but determined that insufficient data existed to make any definitive conclusions. When the PPCP removal performance of a high-purity oxygenated activated sludge plant was compared to a conventional aeration system, the pure oxygen system showed higher removal rates although its SRT was shorter than the conventional aeration plant (i.e., 1 day versus 3 days). In addition, different HRTs operating at similar SRTs had similar removal rates, and therefore suggested that HRT does not significantly affect removal effectiveness in the investigated PPCPs.

Role of Nitrifying Bacteria in Biodegradation

As discussed above, longer SRTs allow for the establishment of slow-growing nitrifying bacteria (i.e., ammonia oxidizing bacteria and nitrite-oxidizing bacteria). Several studies evaluated whether nitrifying bacteria improve the biodegradation of certain emerging contaminants. Major findings from some of these studies are discussed in this section.

The WERF (2005) technical brief indicated that secondary biological treatment that includes nitrification, nutrient removal, and disinfection may remove more than 90 percent of certain steroids, and >95 percent of alkyl phenols; whereas, secondary biological treatment without nitrification and disinfection may decrease removal of these by more than 15 percent. Batt et al. (2006) investigated the role of nitrifying bacteria in activated sludge in the biodegradation of two pharmaceuticals, iopromide and trimethoprim.

The biodegradation of these compounds was conducted in two lab-scale bioreactors using biomass from a stage-2 activated sludge WWTP (operated at an SRT of 49 days). In one of the bioreactors, nitrification was not inhibited (Batch-1 reactor); in the other, nitrification was inhibited with allylthiourea (Batch-2 reactor). Monitoring was also conducted in the WWTP and compared to results obtained from the batch reactors. Both reactors exhibited high removal rates for iopromide; however for trimethoprim, Batch-1 showed a high removal rate of 70 percent, contrasted to the Batch-2 reactor removal rate of approximately 25 percent with nitrification inhibited. Removal rates within the treatment plant, however, were consistent for both pharmaceuticals, showing significantly higher removal rate after nitrification (approx. 60 percent for iopromide and 50 percent for trimethoprim) compared to activated sludge treatment only (<1 percent for both).

Based on these results, Batt et al. (2006) concluded that nitrifying bacteria have a key role in the biodegradation of pharmaceuticals in WWTP that are operated at higher SRTs. This conclusion is supported by Marttinen et al. (2003), who investigated the fate of phthalates in a WWTP with nitrogen removal and observed that about one third of the removal occurred in the nitrification/denitrification treatment phase.

Studies by Yi and Harper (2007), Khunjar et al. (2007), and others have focused on the mechanisms of estrogen removal during nitrification. Possible mechanisms include sorption of estrogens to solids and biotransformation within the treatment facility, especially in the presence of nitrifying activated sludges (Khunjar et al., 2007).

Ammonia oxidizing bacteria have monoxygenase enzymes for ammonia oxidation and these enzymes have been shown previously to be nonspecific and able to accomplish cometabolic degradation of recalcitrant organics.

Cometabolic degradation is a reasonable hypothesis for estrogen degradation because this compound is present at low ng/L concentrations that are below those expected to support microbial growth on that compound alone. One goal of the Yi and Harper (2007) study was to establish whether biotransformation of EE2 is due to cometabolic activity. They conducted batch experiments using enriched cultures of autotrophic ammonia oxiders. Their study and others (Vader et al., 2000, Shi et al., 2004, as reported in Yi and Harper, 2007) showed a strong relationship between nitrification and EE2 removal in enriched nitrifying cultures. Based on batch tests with and without a nitrifying bacteria inhibitor, they concluded that EE2 biotransformation can be cometabolically mediated in bioreactors that are enriched for autotrophic nitrifiers. However, Yi and Harper (2007) noted that the heterotrophic microorganisms, if present in activated sludge processes, may also be responsible for some micropollutant biotransformations. Further work is needed in this area as these tests did not identify the EE2 degradation product to confirm cometabolic degradation and the role of heterotrophs was not accounted for in some tests.

The focus of a Khunjar et al. (2007) study was to identify the role of ammonia oxidizing bacteria compared to heterotrophic bacteria in the biotransformation of EE2. They used pure cultures of ammonia oxidizing *Nitrosomonas europaea* and heterotrophic cultures that were enriched with monooxygenase and dioxygenase enzyme systems. Nitrifying activated sludge mixed liquors were taken from two WWTPs to seed the cultures. EE2 concentrations were 10-15 µg/L. The results of their study showed significant sorption of EE2 to the predominantly heterotrophic culture but none to the *N. europaea* culture.

In addition, biotransformation of EE2 was significant in the *N. europaea* culture. They observed three major EE2 metabolites at different phases of *N. europaea* culture growth that suggest differential action on each byproduct by the nitrifying bacteria; however, additional work is needed to identify these byproducts. The authors also noted that additional research is needed with continuous flow cultivated *N. europaea* to determine whether these metabolites are likely to be present in nitrifying activated sludge. Also, *N. europaea* was not significantly inhibited at EE2 concentrations at or below 10 µg EE2/L, suggesting that ammonia oxidation may not be significantly impacted by concentrations of EE2 that may be typical of those found in the environment.

Use of Reverse Osmosis to Improve Removal Efficiencies

Several studies describe the effectiveness of RO in the removal of PPCP and EDCs from secondary wastewater effluent. Braghetta et al. (2002) calculated the removals rates that could be achieved with a RO step following tertiary treatment for 17 PPCPs. They estimated removals to be > 90 percent for most of the selected compounds. Lower removal rates were estimated for diclofenac (55.2 to 62 percent), ketoprofen (64.3 percent), and paraxanthine (73.7 percent).

As previously discussed, the WERF (2005) technical brief evaluated RO removal rates for several compounds. Specifically, the WERF brief cites numerous studies in which RO achieved removal rates of 90 percent or better for naturally occurring and synthetic steroids, organohalides, metals /organometals, and alkyl phenols.

In addition, Oppenheimer et al. (2007) found that RO was effective in removing all 20 investigated PPCPs below the detection limit including those that were not consistently removed at SRTs of 30 days (i.e., galaxolide) using conventional activated sludge treatment or media filtration.

Hand Compositing

Hand compositing is a series of time proportional grab samples which are collected and composited by hand. Provided the sample volumes are equal and are collected at even intervals, the results should be the same as if done by an automatic sampler (i.e., flow proportional composite sampling). A specific instance where this sampling method may be used is in metal plating shops which have batch discharges from the treatment tank. Provided the tank contains a homogeneous mixture, a minimum of four grab samples are taken of equal amounts and at evenly spaced intervals of time during discharge, to accurately represent the entire tank.

This should represent the waste characteristics of the entire batch discharge to the sewer. One hand composite per batch discharge would be equivalent to a 24-hour composite sample taken at other types of facilities. The sampling data would be compared with the average daily categorical standards or local limits where applicable.

Parshall Fume and Ultrasonic Flow Meter. Here is a great trick if you do not have a stand for your ultrasonic probe, simply use a reflective street cone to hold your probe. Notice the debris and most POTW's will write a NOV for not maintaining the flume and/or uncleanness.

POTW's Wastewater Samples

General
There are four types of samples that are collected by the POTW's Sampling Section: grab, time proportional composites, flow proportional composites, and hand composites. The sampling method used depends largely on the types of analyses to be run, and the nature of the wastestream being sampled. Each sampling method is described in this section.

Most POTW's will define the sampling methods which must be used by industrial users (**IUs**) to obtain representative samples to show compliance with their permits: ***Example***

(1) A grab sample is an individual sample collected in less than 15 minutes without regard for flow or time of day. pH, cyanide, oil and grease, sulfide, and volatile organics must be collected as grab samples.

(2) 24-hour flow proportional composite samples where feasible. The POTW may waive this requirement if the IU demonstrates that this method is not feasible. Samples would then be taken by means of time proportional composite sampling methods or by hand composite where the IU can demonstrate that this will provide a representative sample of the effluent being discharged.

The volume of sample to be collected by any of these methods is dependent on the number and types of analyses that must be performed.

Wastewater Grab Samples
Grab samples are individual samples collected in less than 15 minutes without regard to flow or time of day. Grab samples are normally taken manually, but can be pumped. Oil and grease samples and purgeable organics are exceptions and must be taken manually.

A grab sample is usually taken when a sample is needed to:

(1) Provide information about an instantaneous concentration of pollutants at a specific time.
(2) Quantify the pollutants in a non-continuous discharge (e.g., batch discharge).
(3) Corroborate composite samples if the waste is not highly variable.
(4) Monitor parameters not amenable to compositing such as pH, temperature, dissolved oxygen, chlorine, purgeable organics and sulfides, oil and grease, coliform bacteria, and sulfites.

Collecting Procedure for Water/Wastewater Grab Samples
Policy Example

Lower dipper or mouth of the bottle into water just below surface. In some cases, you will need to rinse the bottle or dipper three times in the sample before obtaining the sample.

Retrieve collected sample to clean processing area.

Rinse the outside of the bottle 3 times to remove contamination.
Pour the sample into the required laboratory bottle.

Filtering (for ortho-P and NOx samples)
You may need to filter the sample; this is true with some water and wastewater samples. Some surface water virus samples need to be filtered.

- Secure caps tightly.
- Bottle preservation is performed in the truck or lab before sampling.
- Secure sample container caps tightly.
- Label the sample containers and place them in an iced cooler before storage.

Timed Composites
Timed samples are usually taken in instances where the intention is to characterize the wastes over a period of time without regard to flow, or where the flow is fairly constant. Timed composite samples consist of a series of equal volume grab samples taken at regular intervals. Usually the interval is 15 minutes with a maximum sampling duration of 24 hours.

However, other intervals can be used and may be more appropriate under some circumstances. Samplers are available which can take up to 10 discreet samples per bottle, for a total of 240 discreet samples. The sampler may be programmed to take any number of samples into one composite bottle which has a 2.5-gallon capacity.

Flow Proportional Composites
Flow proportional composite samples consist of: a series of grab samples whose volumes are equal in size and proportion to the flow at the time of sampling. Samples are taken at varying time intervals, or continuous samples taken over a period of time based on the flow. Wherever possible, flow proportional sampling is recommended because it most accurately reflects the nature of the wastestream.

Equal volume samples taken at varying time intervals are most often collected by the sampling inspectors. A flow measuring device should be used in conjunction with the automatic sampler.

This sampling method is used for all sampling activities except for instances where grab samples are required or time proportional sampling is more expedient and can provide the same accuracy as flow proportional sampling (i.e., constant flow levels).

Pre-Sampling Procedures *Example*

To ensure acceptable analytical results, numerous steps must be followed before a sampling program can be initiated:

(1) Sampling equipment must be clean and in good working order.
(2) Sampling site must be selected.
(3) Types of analyses must be determined.
(4) Proper sample containers must be selected and prepared.

Wastewater Sampling Equipment
The POTW may use one or more of the following portable samplers, ISCO Ultra-Sonic flow meters, SIGMA Depth Sensor samplers, and SIGMA pH Probe samplers. Safety equipment and other necessary equipment are also used.

The equipment that is kept in the sampling vehicle is dependent on the types of sampling activities planned each week, while the equipment stored in the storeroom is for back-up needs and future sampling demands.

Each sampling vehicle should be equipped with at least one sampler and one flow meter more than is needed for the particular sampling period. For example, three scheduled flow proportionate sampling sites would require a vehicle to be equipped with four samplers and four flow meters. At least one spare battery for each type of equipment taken into the field should also be placed in the sampling vehicle.

Ancillary equipment, such as supports, harnesses, blowers, etc., that must be carried in each vehicle will depend on the nature of the sampling location.

In order to keep the equipment in good working order, it should be maintained and cleaned on a regular basis. Routine maintenance and cleaning procedures should be written into the procedures.

Sampling Equipment Maintenance Policy Example
Basic maintenance for samplers includes: periodic calibration, general equipment checking, and replacement of the internal desiccant and fuses. Routine cleaning should be done as covered in SOP or equivalence.

Basic maintenance of the flow meters includes: periodic replacement of the internal desiccant, plotter paper, ribbon, fuses, and any broken re-roll spool assemblies. **Note**: on this assembly there are two tabs on the sides of this piece which are extremely thin and easily broken.

The NiCad and Gel Cell batteries need to be recharged on a regular basis.

Any battery that reads less than 12.50 when checked should not be installed or left on any of the sampling equipment. At the battery charging station, areas are set aside for batteries that need to be charged and batteries already charged.

To prolong battery life, batteries should be charged for a maximum of 24 hours, in accordance with the procedures described in the manufacturer's operations and maintenance manuals.

It is important to note that charged NiCad batteries, if left unused for a long time, are nevertheless slowly discharging.

Gel cell batteries are generally more stable. Voltage readings should be taken **before** the charged batteries are taken into the field to be sure that they still have a full charge.

When a sampler, flow meter, or ancillary equipment needs more specific repairs, the manufacturer representative should be contacted and arrangements made for repair or replacement of the equipment.

Cleaning Automatic Samplers Policy Example
Samplers, sample jars, grab beakers, and all other equipment used in collecting samples must be cleaned between their uses at each site, to avoid the possibility of cross contamination. Latex or nitrile gloves or equivalent should be worn to protect against infections and acid burns. The following steps should be taken to ensure the proper cleaning of the sampling equipment.

(1) Break down the sampler and lay the three components in a row.

(2) Place the strainers and weights in a plastic bucket.

(3) Set the glass composite jars and Teflon caps off to the side, to be cleaned separately from the samplers.

(4) Pour a small amount of diluted (1:128) O-Syl disinfectant and MICRO soap into each sampler component, the bucket containing the strainers and weights, and the composite jars.

(5) To clean the sampler components:

- (a) Partially fill the sampler bases and cover with water.
- (b) Use a brush to scrub the inside and outside of each sampling component. Using a small bottle brush, thoroughly scrub the inside of the intake tube and the float housing of the sampler head (these are critical areas since they come in contact with the sample).
- (c) Rinse off the soap with fresh water.
- (d) Stack each component so that it will dry quickly and thoroughly.
- (e) Reassemble the sampler after the components are dry, and store it in the proper compartment of the sampling van. Leave the sampler lid loose so moisture won't be trapped.
- (f) Clean the strainers and weights in the bucket. Empty the contents of the bucket and rinse the bucket, strainers, and weights. After they have dried, place them in the proper storage areas of the sampling van.
- (g) Drain the wastewater tank of the sampling van into the sewer drain.
- (h) Refill the fresh-water tank on the sampling van with potable water.

Sampler Bottle Cleaning and Preparation Policy Example

(1) Fill each jar with O-Syl (same dilution as used in the sampler disinfection), MICRO soap, and fresh water.

(2) Thoroughly scrub the inside and outside of the jars until they are sparkling clean. Make sure that all oil and grease are removed.

(3) Rinse the jars with fresh water.

(4) Pour a small amount of 1:1 nitric acid into one jar, and securely place the proper Teflon cap on the jar. Swirl the nitric acid throughout the jar, remove the lid, and pour the nitric acid into the next jar. Repeat this procedure until all the bottles have been treated. Rinse bottles with water after the acid wash. **NOTE:** Wear safety glasses or a full-face shield to protect your eyes.

(5) Place jars in the drying oven. If jars are to air dry use Acetone to clean the bottles the same way as stated in (4) above. Let the jars and caps dry completely.

(6) Place the jars, with their caps on loosely, in their respective places on the sampling van.

Selection of Pretreatment Sampling Site
In order to ensure the collection of valid samples, a representative sampling site must be selected. For industrial sampling, the sites are designated in the permit.

Industrial Users - Permitted/Non-permitted Example
The sampling points within an industry vary with each industry depending on the nature of the process and location of pretreatment facilities. Therefore, exact locations must be identified on a case by case basis. However, the following general principles apply in all cases:

(1) A permanent sampling location(s) must be identified for use by the POTW and the IU.

All permitted industries are required to install a sampling vault. The location of the vault is designated by the enforcement inspector. The enforcement inspector responsible for an individual company or site is responsible for providing directions (**maps**) of the specific sampling points, as well as current copies of permits and the name of the contact person and phone number. This information needs to be kept current in the sampling file. Locations of sampling points need to be compared to what is listed on the currant permit. If sampling points that the POTW is using do not agree with permit location, do not sample - refer to Chief Inspector or Supervisor.

(2) The sampling location should be easily accessible and relatively free of safety hazards.

(3) For categorical industries, there should be, if possible, no discharge present other than that from the regulated process.

If other wastestreams are combined with the regulated wastestream prior to the sampling location, the combined wastestream formula will need to be utilized. The sampling crew must be aware of lower limits to correctly show analysis on chain of custody.

(4) If the rate of industrial process discharge flow is needed (i.e., where mass limitations are applied), the sampling location will need to be located where the flow of the wastestream is known or can be measured or estimated and flow rates for the other wastestreams obtained.

(5) In instances where sampling must be performed in the sewer outside of the building, the IU must install a sampling vault in accordance with Code.

Sample Type and Analyses
Different sample volumes are required for various analyses. In addition, the laboratory has developed standard volumes for routine analyses performed on industrial waste samples as follows:

(1) BOD/COD/TSS (1000-2000 ml, plastic) or equivalent
(2) Heavy metals (500-2000 ml, plastic) or equivalent
(3) Cyanide (2000 ml, plastic) or equivalent
(4) Oil and grease (1000 ml, level-one glass) or equivalent

Selection and Preparation of Sample Containers
The selection of a sample container is based on the parameter to be measured. The inspector should be familiar with the type of sampling containers and preservatives that are needed.

It is essential that the sample containers be made of chemically resistant material, and do not affect the concentrations of the pollutants to be measured.

In addition, sample containers should have a closure (i.e., leak proof/resistant, Teflon lined) that protects the sample from contamination and be properly labeled before leaving the sampling site.

Wastewater Sample Preservation
Wastewater usually contains one or more unstable pollutants that require immediate analysis or preservation until an analysis can be made.

Sample preservation is needed for composite samples, for example, which may be stored for as long as 24 hours prior to transferring them to the laboratory.

Recommended preservatives and holding times that should be used for specific pollutants are presented in the front of this section.

Quality Assurance/Quality Control Policy *Example*

Quality Assurance/Quality Control (**QA/QC**) measures taken by the sampling crew include equipment blanks, trip blanks, split samples and duplicate samples. Equipment blanks and trip blanks are routine QA/QC measures.

Split samples are taken for Local Limits (pretreatment) sampling and when requested by an industry or laboratory. Split samples requested by an industry are analyzed by their lab at their expense.

Duplicate samples should be run when requested by a Supervisor or Project Leader.

The laboratory prepares all trip blanks/travel blanks used by the sampling crews. This is performed in the laboratory rather than in the field in order to assure that there is no field contamination in the blanks.

Any contamination detected in the blanks would result from field exposure which could in turn affect collected samples.

Chain-of-Custody
Documentation of all pertinent data concerning the collection, preservation and transportation of samples is critical to the overall success of the Wastewater Sampling Program. If sampling is performed for the Pretreatment program, any sampling data may be used as evidence in court proceedings against a noncompliant industrial user. In this case documentation becomes critical. This form is a legal document and is of major importance in a court hearing. Specific procedures with regard to chain of custody are outlined below:

(1) The sampling crew takes a sufficient supply of pre-numbered Industrial Waste Lab Reports, (custody forms) and sample containers into the field.

(2) The sampling crew fills in the sampling form at the time of sample collection, and returns the form to the lab along with the collected sample. Specific information to be completed on the form includes:

(a) CODE: The company ID number assigned by supervisor.

(b) SITE No.: The sampling point ID number assigned by supervisor.

(c) DATE SAMPLED: From - Date sampling began To - Date sample is pulled. If it is a grab sample, only the date the sample was taken will be entered with the other line crossed out.

(d) SUBMITTED BY: This will have a preprinted truck number. The sampling crew will write in their initials on the blank line which follows.

(e) LABEL: A letter is checked and the type of analysis to be performed.

(f) PRESERVATIVE: The method of preservation used. See preservation section to see which preservatives to use.

(g) TYPE OF SAMPLE: Check off whether proportional, timed composite, hand composite, flow or grab sample.

(h) TIME: The time frame needed for collection of the sample. A starting time for sample collection, an ending time, and a total time in hours and quarter hours is recorded, such as 23.25 hours. On a grab sample only, the end time, which is the time the sample was taken, will be entered and the other two lines will be crossed out.

(i) RELINQUISHED BY: This is the signature of person that relinquishes sample to lab personnel, or to any other person taking custody of the sample.

(j) DATE: Date sample is submitted to the laboratory or relinquished to another person.

(k) NOTES TO LAB: Includes any special notes to the lab, such as special analysis required of the sample, a letter code which is assigned to the entity being tested the amount of flow if sample is flow proportional, grab sample pH and temperature, and/or actual sample temperature.

(l) FIELD TEST: Results of any field tests including sample pH, hexavalent chromium, dissolved sulfides, copper, and residual chlorine.

(m) RESULTS: The appropriate box(es) need to be checked to correspond to the label designation chosen above.

(3) When the sampling is completed at a site, the sampling crew labels the bottles with the label letter designation. The samples are sealed with chain of custody seals and placed in an ice chest for transportation to the lab.

(4) The sampling crew submits the samples and the chain of custody form to the laboratory.

(5) The laboratory logs the samples and assigns a Lab Reference Number to the sample. The sample is tracked by means of this number.

(6) Laboratory personnel sign and date the form, and return it to the sampling crew who makes two copies of the form. One copy is for the sampling crew files and the other is for data entry. The original form is returned to the laboratory. It is also important to note that the sampling vehicle should be kept locked at all times when the sampling crew is not in the vehicle, or in full view of the vehicle.

Equipment Maintenance Cleaning Techniques *Example*

It is important to keep all equipment used for sampling clean to reduce the risk of cross contamination.

Is your automatic sampler and equipment clean?

All components of an automatic sampler need to be clean prior to setup, since contamination can occur.

Automatic sampler - Each section should be clean, especially the inlet tube.

Intake tubing - Cleaned or new Tygon or Teflon should be used according to the samples you are collecting.

- Intake line strainer - Clean or stainless steel or Teflon.
- Pump tubing - Clean or new medical grade silicone tubing.

Composite bottle - Generally glass is used for this and it's extremely important that it is clean.

Automatic sampling equipment - all components of an automatic sampler should be cleaned using phosphate free soap and rinsed thoroughly prior to setup.

Follow safety procedures (goggles, gloves, etc...) When cleaning equipment!
Composite bottles used in the automatic sampler need to be cleaned prior to setup.

1. Rinse bottle and cap with tap water to remove any residual contaminants.
2. Wash bottle and cap with a phosphate free soap and tap water.
3. Rinse bottle and cap with tap water to flush off any soap.
4. Add 1:1 nitric acid to bottle, cap and shake to cover entire inside of bottle and cap. Pour out 1:1 nitric acid.
5. Triple rinse bottle and cap with analyte free water.
6. Allow bottle and cap to dry.
7. Store Bottle with cap loosely screwed onto bottle to keep contaminants out.

Tubing - clean or new
Using clean tubing (intake and pump) for each sampling event will eliminate the need to clean the tubing. If tubing is not going to be changed before a sampling event, then the tubing will need to be washed.

1. Pump clean tap water through tubing, using the automatic sampler pump to transfer water from one container to another.
2. Place tubing into another container that has a phosphate free soap in it and pump the soapy water through the tubing 3 or 4 times while catching the discharge in a separate container.
3. Pump clean tap water through tubing to rinse out soap.
4. Add 1:1 nitric acid to clean tap water and pump this solution through tubing 3 or 4 times.
5. Pump analyte-free water through tubing until all residual acid has been removed.
6. Keep tubing in a clean place until next sampling event.

Regular Maintenance Includes the Following

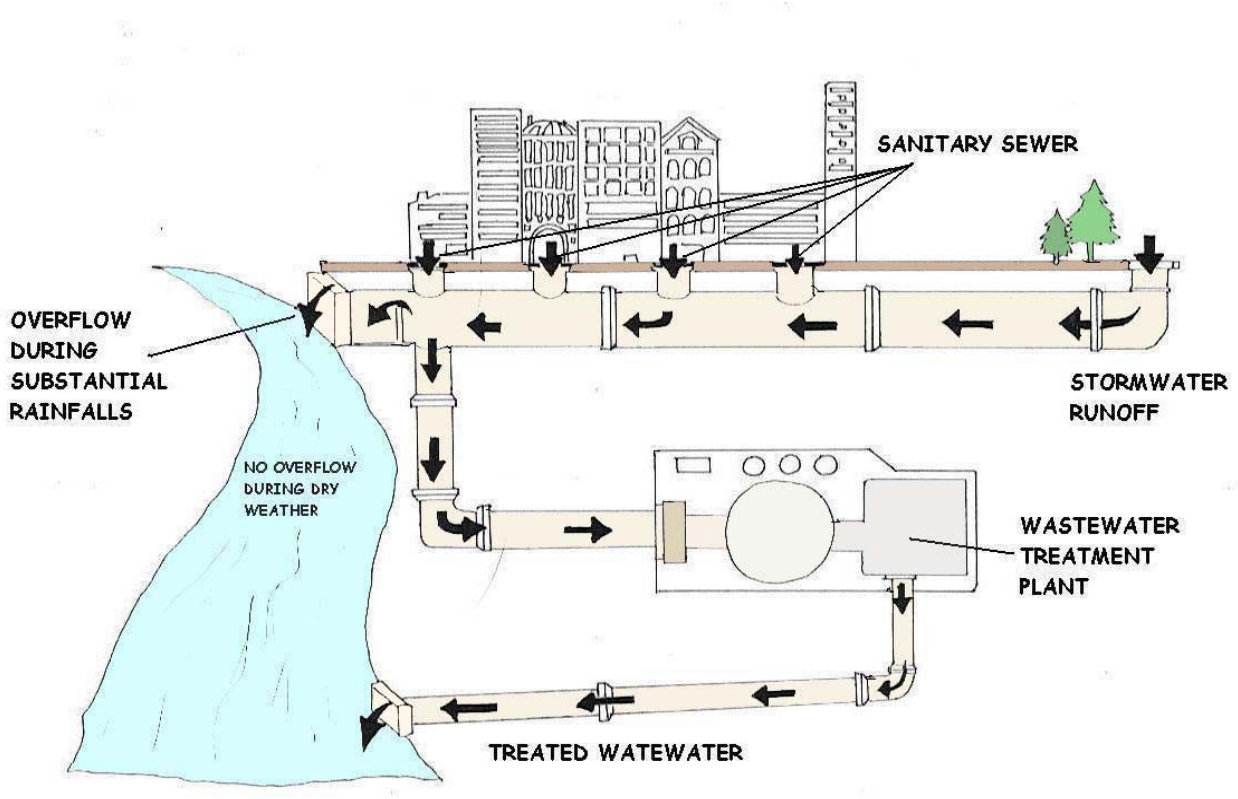
Automatic Sampler

- Washing
- Drying
- Change desiccant
- Check full bottle shut-off float
- Recharge battery

Flowmeter

- Washing
- Drying
- Change plotter paper
- Change printer ribbon
- Change desiccant
- Recharge battery

Record keeping - keep a record of cleaning dates. What was cleaned, and when.



A modern wastewater treatment facility may have up to 10 different sampling sites some are necessary for compliance purposes, some sites are to please certain agendas or for political purposes. These sampling sites may include Local Limits, QA/QC, Influent, Outfall, Chlorine residual, and many more sites to maintain compliance and to ensure that the plant is running efficiently.

Proper Sample Handling

The proper handling of water quality samples also includes wearing gloves. Gloves not only protect field personnel, but also prevent potential contamination to the water sample. Always wear powderless, disposable gloves. When sampling for inorganics, wear latex gloves. Nitrile gloves are appropriate for organics.

The following sections provide a field reference for chain of custody procedures, sampling surface water and ground water, and further provides procedures for measuring field parameters and handling water-quality samples.

Use chain-of-custody procedures when coolers and containers are prepared, sealed and shipped. They will remain sealed until used in the field. When making arrangements with the laboratory, make sure you request enough containers, including those for blank and duplicate samples. Order extra sample bottles to allow for breakage or contamination in the field.

Some samples require low-temperature storage and/or preservation with chemicals to maintain their integrity during shipment and before analysis in the laboratory. The most common preservatives are hydrochloric, nitric, sulfuric and ascorbic acids, sodium hydroxide, sodium thiosulfate, and biocides. Many laboratories provide pre-preserved bottles filled with measured amounts of preservatives. Although most federal and state agencies allow the use of pre-preserved sample containers, some may require either cool temperatures or added preservatives in the field.

When the containers and preservatives are received from the laboratory, check to see that none have leaked. Be aware that many preservatives can burn eyes and skin, and must be handled carefully. Sampling bottles should be labeled with type of preservative used, type of analysis to be done and be accompanied by a Material Safety Data Sheet (**MSDS**).

Make sure you can tell which containers are pre-preserved, because extra care must be taken not to overfill them when collecting samples in the field. Check with the laboratory about quality control procedures when using pre-preserved bottles. Coolers used for sample shipment must be large enough to store containers, packing materials and ice. Obtain extra coolers, if necessary. Never store coolers and containers near solvents, fuels or other sources of contamination or combustion. In warm weather, keep coolers and samples in the shade.

Field Parameters
Measure and record the field parameters of temperature, electrical conductivity, pH and dissolved oxygen in an undisturbed section of stream flow. Other parameters may be measured, if desired.

Water Sample Station commonly found at most water or wastewater treatment plants. This tap will allow the operator to obtain Grab Samples for pH, Temperature, COD, Bacterial, ORP, OUP, Organics and Inorganic field parameters.

pH Meter pH scale is between 0-14 0 being Acid and 14 being Base. For fun, we measured Orange Juice and that is pH 3.5 or so - acid! We also measured Welch's Grape Juice and the meter showed 3.4 - also an acid (and slightly stronger). Here is a close-up of the pH41 meter we use and its pH 7.0 buffer solution (used for calibration and storage - there are a few drops of the buffer solution in the cap of the pH41 to keep it properly moist when not in use).

QA/QC Field Procedures for Plant Sampling *Example*

Duplicate Sampling Procedure
The purpose of Duplicate Samples is to check the laboratory's ability to reproduce analytical results. Duplicate Samples are to be collected using these steps:

1. Determine amount of sample needed. If a flow proportion sample is required, then base the amount of sample needed on the current flow reading. If a flow-proportion sample is not required, then use the predetermined amount for the sampling site.
2. Collect sample using a grab type sampler or a sampling head.
3. Measure the amount determined in Step 1 using a graduated cylinder or other accurate measuring device.
4. Pour measured sample into sample container that is not marked as the Duplicate Sample.
5. Measure same amount as in Step 1.
6. Pour second measured quantity into sample container marked for Duplicate Sample.
7. Process both samples using standard procedures and submit both samples to laboratory.

Split Sampling Procedure
The purpose of Split Samples is to check analytical procedures by having the samples analyzed by two different laboratories. Split Samples are to be collected using these steps:

1. Determine amount of sample needed. If a flow proportion sample is required, then base the amount of sample needed on the current flow reading. If a flow-proportion sample is not required, then use the predetermined amount for the sampling site.
2. Collect sample using a grab type sampler or a sampling head.
3. Measure the amount determined in Step 1 using a graduated cylinder or other accurate measuring device.
4. Pour measured sample into sample container that is not marked as the Split Sample.
5. Measure same amount as in Step 1
6. Pour second measured quantity into sample container marked for Split Sample.
7. Process both samples using standard procedures and submit both samples to the laboratory. The laboratory will be responsible for submitting the samples to the outside laboratory that will be analyzing the Split Sample.

Trip Blank Procedure
The purpose of Trip Blanks is to determine if the sample bottles have been adequately cleaned, and if sample contamination occurs between the time sample bottles leave the laboratory to the time that samples are returned to the lab.

Trip blanks are prepared by the laboratory using bottles supplied by the sampler. They are picked up by the person who begins the sampling day. Trip blanks are placed in the cooler which contains the other samples and remain there until the samples are turned into the laboratory.

A normal day for a WWT sampler. Here she is looking up a flow rate to determine the volume of wastewater that is flowing. Many samplers do not know simple math formulas to determine flow and rely solely upon the electric measuring devices. These measuring devices can fail or be programmed incorrectly, or batteries can die. You better be prepared for the worst case scenario. Pickle bottle is shown in the middle photograph.

Field Equipment Blank Procedure

The purpose of Field Equipment Blanks are to test the procedure for cleaning the sample measuring container to determine if cross contamination between sample sites has occurred. These Blanks are needed only at sites where flow-proportion samples are taken. Follow these steps when collecting a Field Equipment Blank:

1. Collect Field Equipment Blank **AFTER** collecting a sample and **BEFORE** moving to the next sampling location.
2. After collecting sample, triple rinse sample measuring container, usually a graduated cylinder, using High Purity water.
3. Open a sealed bottle of High Purity Water.
4. Pour the High Purity Water into the sample measuring container that was just rinsed.
5. Pour the High Purity water from sample measuring device into sample bottles labeled for the Field Equipment Blanks.
6. Repeat Steps 3 through 5 until all Field Equipment Blank sample bottles have been filled.
7. Process samples using standard procedures and submit to laboratory.

An equipment blank is high purity water which has been collected in a composite sample bottle or a series of discrete bottles from an automatic sampler. Equipment blanks are used to evaluate the reliability of composite samples collected in the field.

The data produced from the equipment blank indicates the performance of the sample collection system, which involves the cleaning of sampling equipment, and accessories, preservation techniques, and handling of samples.

The objective is to demonstrate that the samples are not contaminated by inadequate cleaning of equipment, contaminated preservation additives or sample collection techniques, and to provide documented records on Quality Assurance Practices.

Procedures to be followed in collecting the equipment blanks are outlined below.
(Also see QA/QC check list, example).

(1) The sampler is to be assembled completely in the manner determined by the parameters the crew will be sampling (i.e. if sampling for organics, Teflon suction tubing must be used at that site). The composite jar inside the sampler must always be rinsed out thoroughly with high purity water.

(2) Program the sampler to collect the proper amount of high purity water that is representative of the sample parameters that will be collected at that site. Grab samples are excluded. Pump high purity water through the strainer and intake tubing prior to filling the sampler bottle. Then, place the strainer into as many fresh, uncontaminated bottles of high purity water as needed to collect the necessary volume of sample.

(3) If the sampler is set up in the discrete mode, the crew must then transfer the collected samples into the field composite bottle and shake to mix thoroughly.

(4) Transfer the sample from the field composite bottle into its respective lab sample bottles. Test and preserve the samples as appropriate for the parameters being analyzed.

(5) Follow the chain of custody procedures outlined in SOP for turning the samples in to the laboratory. All paperwork must be completed at this time, and all bottles must be marked accordingly. Custody seals must be used. The crew must note the sampling activity in a logbook that is kept specifically for documenting preparation of equipment blanks and/or any other QA activities.

Wastewater Sampling Procedures/Techniques

General Guidelines

In general, the following guidelines should be observed in conducting sampling activities:

(1) Samples being collected must be representative of the wastestream being tested.

(2) Samples shall be collected in uncontaminated containers and preserved properly.

(3) Samples should be of sufficient volume for the required analyses.

(4) Samples should be stored in a manner which does not alter the properties of the sample prior to chain of custody transfer.

(5) Samples should be properly and completely identified by marking them with the proper information.

(6) Sample lines should be as short as possible and the smallest practical diameter to facilitate purging, reduce lag time, and give adequate consideration to maximum transport velocity. Also, they should have sufficient strength to prevent structural failure.

(7) Sample lines should be pitched downward at least 10 percent to prevent settling or separation of solids contained by the sample.

(8) Samples should be delivered as quickly as possible to the laboratory.

Specific Techniques
Sampling techniques in addition to the above general guidelines must also recognize differences in sampling methodology, preservation, and analytical methods.

The following sections specify techniques that differ by pollutant group and discuss such factors as sampling methodology (e.g., composite, grab, etc.), type of container, preservation and holding time.

Sampling Techniques for Volatile Organics

Volatile organics are analyzed in accordance with EPA methods 601, 602, and 603.

Due to the volatility of these compounds, only grab samples can be taken. If a composite sample is needed, individual grab samples must be collected and composited in the laboratory prior to analysis.

The procedures that must be followed in taking these samples are outlined below.

NOTE: Gloves, clothing, face, and eye protection must be worn when handling volatile organics.

In addition, the sampling crew must thoroughly clean those parts of the body that have been exposed to these materials.

(1) For each sampling date, the lab will also provide two additional bottles to be used as a backup in case of breakage. These sampling vials are only good for one week. If any are unused, they must be returned to the lab for disposal.

(2) The lab will provide one sample trip blank per sampling date. This bottle is to be kept on ice until the samples are submitted to the lab. At least one day prior to sampling, go to the lab and request the sample bottles (40 ml vials) for the specific sampling site, as indicated by the sampling plan. The laboratory will arrange to have the appropriate number of sample bottles prepared, based on the number of analyses to be performed. The sampling crew should make sure that all bottles are provided for these samples by the lab technicians.

(3) Collect the sample in a clean glass beaker. Test for chlorine with the Hach test kit. If there is any chlorine residual, neutralize the chlorine with sodium thiosulfate ($Na_2S_2O_3$) and retest for chlorine. Repeat until there is no chlorine residual. Make notes on chain of custody sheet if extra amounts of sodium thiosulfate are required for neutralization.

(4) Remove the vials from the ice. There will be two empty vials for the 601 sample and two vials with HCl for the 602. The HCl will already have been measured into the vials by the lab personnel.

(5) Fill the vial to just overflowing in such a manner that no air bubbles pass through the sample as the vial is being filled. This is accomplished by pouring the sample from the beaker into the vial along the side of the vial to minimize the possibility of entrapping air in the sample. Do not rinse out or overfill the vials, this will wash out the preservative in the vial.

(6) Seal the vial so that no air bubbles are entrapped in it. Remember to put the Teflon side of the cap facing down onto the vial.

(7) To be sure there are no air bubbles, turn the vial upside down and tap it against the palm of the hand. Check to see if there are air bubbles along the sides or bottom of the vial. If there are bubbles,

unseal the vial, top off the vial, and reseal. Check the vial again for the presence of bubbles.

(8) All samples must be maintained at four degrees centigrade from the time of collection until the time of extraction. Custody seals must be placed on all samples, and all paper work must be filled out properly.

(9) Return the sample bottles and QA/QC bottles to the laboratory the same day the sample is collected.

Acid/Base/Neutral Extractable Organics and Pesticides Example

Acid extractable organics are analyzed in accordance with EPA methods 604 and 625. Base/neutral extractable organics are analyzed in accordance with EPA method 625, or individual methods for various groups of compounds including EPA methods 605, 606, 607, 609, 611, and 612. Pesticides are analyzed in accordance with EPA method 608.

The procedures that must be followed in taking these samples are outlined below.

(1) Samples must be collected in certified clean one-gallon amber glass bottles with Teflon lids.
(2) Travel blanks or QA/QC bottles may not be required with the samples.
(3) Grab samples must be collected in amber glass bottles. They do not have to be completely filled, but must be a minimum of 1/3 to 1/2 full. Bottles should not be pre-washed with samples prior to filling.
(4) For composite sampling, glass composite bottles must be used and pre-cleaned. Teflon tubing must be used for the suction piping. The pump tubing must be medium grade silicone rubber.
(5) The composite bottle in the sampler must be kept refrigerated (putting ice in the sampler) at 4 degrees Celsius. If amber glass is not used, (i.e. 2 1/2-gallon clear composite sampler bottle,) the sample must be protected from the light during collection and compositing. The compositing must be done in the field, (i.e. when discrete sampling has been used).
(6) All samples must be iced at four degrees Celsius from the time of collection until extraction.
(7) The sample should be checked for the presence of chlorine using field test kits that provide results in accordance with EPA methods 330.4 and 330.5. If chlorine is determined to be present, 80 mg of sodium thiosulfate should be added to each bottle. The sample must be retested for chlorine. This procedure must be repeated until there is no residual of chlorine shown. The amount of sodium thiosulfate added must be noted on the chain of custody if in excess of 80 mg.
(8) All necessary paperwork must be completed at sampling site. All bottles must be properly labeled, and have custody seal.

Sampling Techniques for Heavy Metals

(1) Generally, all metal samples collected are to be composite samples, i.e., flow/composite, time/composite, or hand composite.

(2) For composite sampling, place the lid on the bottle and agitate the bottle to completely mix the composite sample.

(3) Transfer the required amount from the composite container to either a 500 ml or 2000 ml clean plastic bottle. Check the pH of the sample as described in Section 8.7.2.5.

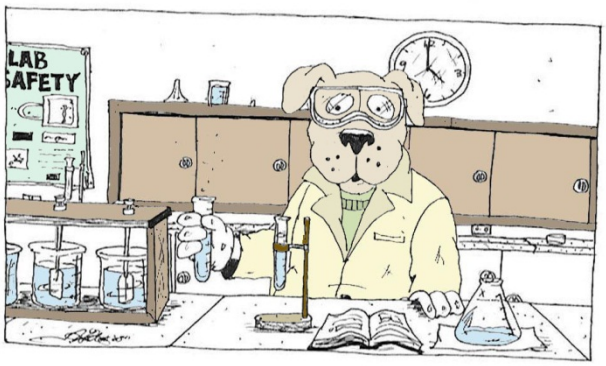
Note: For inductively coupled plasma (**ICP**) metal analysis, a 500 ml clean plastic bottle is required. For extra metals or metals by furnace, a 2000 ml clean plastic bottle is required.

(4) Add nitric acid (1:1 solution) to the sample to reduce the pH to below 2.0. Usually, 2 ml/500 ml is sufficient. Recheck the pH to be sure it is below 2.0. Make a note on the lab sheet if more than two ml of acid is required to bring the pH below 2.0.

(5) Label the sample bottle with the corresponding IW number and proper analysis code letter. Attach the custody seal to the sample, then store in the ice chest until transferred to the laboratory. Fill out the IW lab sheet with all the pertinent information, being careful to include all required parameters and the type of analysis required, e.g., ICP/furnace.

(6) When a grab sample is necessary, rinse out the receiving sample bottle with an aliquot of the sample stream at least three times. Then fill the sample bottle and proceed with steps two through four described above.

(7) When a split sample is requested (i.e., one for the samplers and one for the user), the composite sample is prepared as described in item one. Providing there is sufficient sample, a portion is transferred into the bottle provided by the user.



(8) If more than one site is sampled per day, a clean composite container (i.e., two and one half-gallon glass jar), must be used at each site.

(9) If a discreet sampler is being used, at the time of collection combine all the samples that have been collected into a single clean composite bottle. Then follow the preceding steps one through four, and refer to step six if a split is requested.

Cyanide

To assure that the sample can be analyzed for cyanide, no chlorine can be present in the sample. Procedures for taking cyanide samples are as follows:

(1) This sample is normally a grab sample. The cyanide sample is a composite sample when collected as part of Priority Pollutants or Plant Sampling at the waste treatment plants.

(a) In the sampling file, check the industries' wastewater discharge permit and locate all cyanide (CN) sampling sites. If the sampling sites are located in a confined space, follow Confined Space procedures before collecting the sample or samples.

(b) Collect 2000 ml (maximum), 1000 ml (minimum), of CN sample into a type C plastic bottle.

NOTE: 2000 ml is the standard, but for batch dischargers 1000 ml is adequate.

(c) Test the cyanide sample for pH and temperature with the pH meter. Record the results on the custody sheet (Industrial Waste (IW) lab sheet).

(d) Test for chlorine with **Hach Total Chlorine Test Kit** (the instructions are located in the kit)

(e) If chlorine is present in the CN sample, neutralize it with Ascorbic Acid ($C_6H_8O_6$). For ascorbic acid neutralization, add $C_6H_8O_6$, a few crystals at a time, until five mls of sample in the test tube produces no color. Then add an additional 0.06 g of $C_6H_8O_6$ for each liter of sample volume.

(f) Once all Cl_2 has been neutralized, preserve the sample with Sodium Hydroxide (NaOH) and raise the pH to >12. Verify the >12 pH with a pH meter or pH test strips.

(g) Mark on the side of the CN sample bottle the Lab sheet number (using a water proof marker), and place a corresponding custody seal across the sample bottle tightened cap. Place a Cyanide label on the bottle if cyanide is suspected of being present in the sample.

(h) Store the CN sample in the ice at four degrees Celsius and transport it to the laboratory.

Total Sulfides

(1) The Total Sulfide sample is collected as a grab sample only. Use a clean 500 ml plastic bottle to collect the sample. This sample may be pumped into the sample container or collected directly from the discharge side of the sampling device.

(2) Preserve the sample with 1 ml of 2N Zinc Acetate ($C_4H_6O_4Zn$) and then add Sodium Hydroxide (NaOH) to raise the pH > 9.

(3) Label and seal the sample with a custody seal. Cool to 4°c.

Oil and Grease/TPH

Oil and grease samples are collected as two separate samples:

METHOD 413.1 (Oil and Grease). Non-volatile hydrocarbons: vegetable oils, animal fats, waxes, soaps, and related matters.

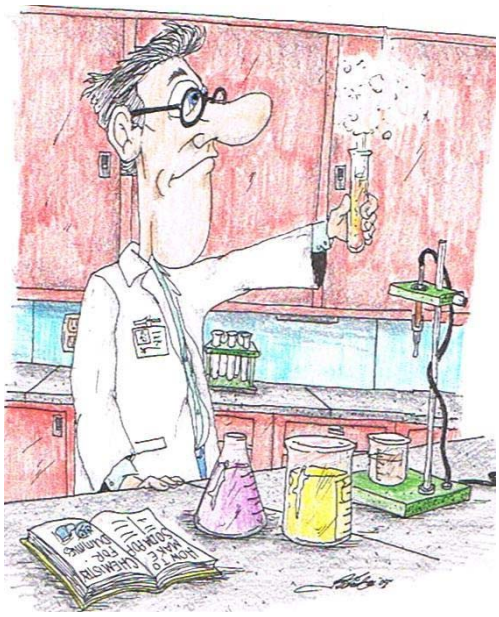
METHOD 418.1 (TPH). Extractable petroleum hydrocarbons: light fuels and mineral oils.

(1) This is a grab sample only. The bottle used to take the sample must be the same bottle given to the laboratory for analysis. Do not pump or transfer the wastewater sample into the bottle. Obtain a level one clean 1000 ml glass bottle, do not use a pre-preserved bottle because you will lose the preservative when collecting the sample.

(2) Collect the sample by placing the bottle neck down (up-side down) into the effluent stream below the surface. This should be as close to the discharge pipe or point as physically possible. Turn the bottle, allowing the bottle to fill, while keeping the bottle below the surface. Remove the filled bottle and cap it. Never skim the surface of the effluent stream.

(3) Preserve the sample using five ml of sulfuric acid (H_2SO_4) for method 413.1 or hydrochloric acid (HCL) for method 418.1 (6:1 Ratio) to a pH of less than two. Reference 42 of methods 418.1 and 41 of methods 413.1. When more than five ml of HCL is used to lower the pH to less than two, make note of how much additional acid is used, and record this on the lab sheet. Also indicate required analyses method on lab sheet.

(4) After making sure the sample is well mixed and preserved, seal and attach the proper identification (custody) label to the bottle. Then attach a custody seal across the lid. Store all samples at four degrees centigrade.



(5) Under no circumstances are Inspectors to collect an oil and grease sample or any other grab sample for IUs.

(6) All samples must be taken from a good representative flow. If there is any question as to whether there is sufficient flow for a representative sample, do not collect any sample. Make the necessary notes in the file report as to why no sample was obtained.

BOD/COD/SS

(1) 24-hour composite sampling is always used for this test. Agitate the bottle to completely mix the composite sample. Do not allow the solids to settle out before you pour off the sample.

(2) When more than one sample is being taken from a composite bottle, the BOD/COD/SS is taken first. The lab needs 1000 ml if the sample is cloudy or has solids. If the sample is clear, you must collect 2000 ml. Transfer the appropriate volume to the sample bottle.

(3) Take the pH/temperature of the sample with either pH paper and a thermometer, or the pH meter carried on the sampling trucks.

(4) Label the sample bottle and place a custody seal over the lid. Store on ice at four degrees centigrade.

(5) Should split samples be requested, they are given when it is sure there is enough sample for POTW's requirements. Users must provide their own sample containers and allow POTW's staff to pour off samples.

Rotating Bar Screens

The wastewater headworks is a key sampling location both for compliance and for process control.

Virus Sampling *Example*

Viruses are microbiological organisms which can cause infectious diseases. Wastewater recharge and sewage disposal into the environment may contribute to the occurrence of viruses in surface water and groundwater. Viruses are the most mobile and infectious of the waterborne pathogens. Large volumes of water must be filtered to detect viruses. This involves passing the water samples through a cartridge filter by use of a gasoline driven pump.

(1) **Equipment Needed**

Most of the equipment required for virus sampling should be available on the sampling trucks. However, some equipment is virus sampling specific. The needed equipment is as follows:

(a) Gasoline/oil powered water pump or equivalent
(b) Hoses - intake (supplied with pump) and discharge (garden type, with female connectors at both ends)
(c) Two 55-gallon plastic containers or equivalent
(d) Filter apparatus
(e) Cartridge filters
(f) Sodium thiosulfate (two 500 gram bottles/site)
(g) Gasoline can with gas/oil mixture
(h) Hach total chlorine test kit
(i) Large plastic Zip-lock bags (supplied with cartridges)
(j) Chain of custody sheets
(k) Thermometer
(l) Water-proof marker
(m) Latex gloves
(n) Liquid bleach
(o) Cooler with blue ice
(p) pH meter

(2) **Sampling Procedure**

Check the pump for gas/oil prior to starting (**Note**: do not fill while it is running). Make sure the gas/oil mixture is correct by checking the mixing instructions on the side of the two-cycle pump oil can. Latex gloves should be worn for protection, and to prevent contamination of the filters.

Connect the hoses and filter housing (with no filter) to the pump, and run the effluent through it for one to two minutes to flush the system. Next, pump effluent into the two 55-gallon drums and rinse them out. (**Note**: If disinfection was not possible after the last sampling, then 50-100 gallons of effluent should be pumped through the entire equipment set up prior to placing the filter in the housing.)

Pump effluent almost to the top (just above the handles) of both containers. While the drums are filling, check the water in the drums for chlorine using the Hach test kit and record the results and the temperature on the custody sheet.

If chlorine is present and needs to be eliminated, add 500 grams of sodium thiosulfate to each container to eliminate it.

After visual observation has determined that all the sodium thiosulfate has dissolved, retest to make sure there is a <0.1 ppm chlorine residual. If chlorine

was removed, take the hose from the channel, allow it to drain, and re-prime the pump with the de-chlorinated water.

Pump this water through the system to flush it, and adjust the flow to fill a one-gallon jug in about 15-20 seconds. Don't waste too much water, as the flow can be adjusted after the filter is inserted. Install the filter into the blue holder, being very careful not to touch it with your hands (wear clean latex gloves). There are two black washers that go with the filter, one on the bottom and the other on the top. Make sure these are aligned with the filter housing to prevent leaking. Screw the holder and filter onto the apparatus.

Refuel the pump, restart it, and adjust the water flow so that it is close to 15-20 seconds per gallon. Make sure the housing doesn't leak. Try to keep this amount of flow, since too great a flow will cause pass-through in the filter. Pump the water from both containers until they are empty.

Stop the pump, remove the filter (wear clean latex gloves), and place it in its original zip-lock bag. The washers do not need to go with the filter, but if they fall into the bag it is better to leave them than take the chance of contaminating the filter trying to remove them.

Fill in the information area on the zip-lock bag with a marker, indicating the plant being sampled and the date, and put it in the cooler with the blue ice provided. The blue ice keeps the temperature at 4 degrees Celsius to prevent significant die-off of the viruses.

While at the site, or later at the plant, mix a half-gallon of bleach to 10 gallons of clean water. Pump it through the flow system and the containers. Rinse everything with fresh water and drain it so it is ready for the next time. Let the pump cool before storing it. Store the gas/oil mixture in the warehouse flammable storage cabinet.

Parasitological Sampling

Parasitological sampling utilizes the same equipment and techniques as in the virus sampling described above. However, a different type of filter, which is provided by the Lab, is used.

Field Tests *Example*

Sampling Procedures for Hexavalent Chromium (Hach Kit)

(1) Rinse out the two color viewing tubes with a portion of the sample to be tested.

(2) Refill one of the color viewing tubes to the 5 ml mark with a sample (this is the test sample). Using the clippers provided in the test kit, open one ChromaVer three chromium reagent powder pillow. Add the contents of the pillow to the sample. Stopper and shake to mix and put the tube in the color comparator.

(3) Fill the other viewing tube with a sample and put it in the left side of the color comparator (this is the blank).

(4) Let the viewing tubes sit in the color comparator for approximately 5 minutes. The samples should not be exposed to direct sunlight.

(5) Hold the color comparator up to a light source and view the two samples through the two openings in the front. Rotate the dial on the holder until the color appears the same in both samples. Record the results from the dial (which is read in mg/l Cr +6) onto the chain of custody form.

Sampling Techniques for Dissolved Sulfides (Chemetrics, Inc. Kit)

(1) Collect a 25 ml grab sample in the container provided.

(2) Add three drops of activator (amber colored liquid) and mix well.

(3) Break a sulfide chemet Type S glass ampule and add the contents to the 25 ml container.

(4) Let stand five minutes.

(5) Take a reading and record the results on the chain of custody form. If the reading is 0.0 then show the results less than 0.1 mg/l.

Sampling Techniques for Free and Total Chlorine (older colorwheel Hach Kit)

Procedures for determining free chlorine are as follows.

(1) Rinse out the two color viewing tubes with a portion of the sample to be tested.

(2) Refill one of the color viewing tubes to the 5 ml mark with a sample (this is the test sample). Using the clippers provided in the test kit, open one DPD free chlorine reagent powder pillow. Add the contents of the pillow to the sample. Stopper and shake to mix and put the tube in the color comparator. All of the powder does not have to dissolve to obtain correct readings.

(3) Fill the other viewing tube with the original sample and put it in the left side of the color comparator (this is the blank).

(4) Let the viewing tubes sit in the color comparator for approximately 1 minute. The samples should not be exposed to direct sunlight.

(5) Hold the color comparator up to a light source and view the two samples through the two openings in the front. Rotate the dial on the holder until the color appears the same in both samples. Record the results from the dial (which is read in mg/l free chlorine) onto the chain of custody form.

Procedures for determining total chlorine are as follows.

(1) Rinse out the two color viewing tubes with a portion of the sample to be tested.

(2) Refill one of the color viewing tubes to the 5 ml mark with a sample (this is the test sample). Using the clippers provided in the test kit, open one DPD total chlorine reagent powder pillow. Add the contents of the pillow to the sample. Stopper and shake to mix and put the tube in the color comparator. All of the powder does not have to dissolve to obtain correct readings.

(3) Fill the other viewing tube with a sample and put it in the left side of the color comparator (this is the blank).

(4) Let the viewing tubes sit in the color comparator for approximately 3 minutes. The samples should not be exposed to direct sunlight.

(5) Hold the color comparator up to a light source and view the two samples through the two openings in the front. Rotate the dial on the holder until the color appears the same in both samples. Record the results from the dial (which is read in mg/l total chlorine) onto the chain of custody form.

COD Reactor

Dissolved Oxygen

Dissolved oxygen (**DO**) in water is not considered a contaminant. However, the (DO) level is important because too much or not enough dissolved oxygen can create unfavorable conditions. Generally, a lack of (DO) in natural waters creates anaerobic conditions. Anaerobic means without air. Certain bacteria thrive under these conditions and utilize the nutrients and chemicals available to exist. *Under anaerobic conditions the reaction is:*

Anaerobic:

Organics-→ intermediates + CO_2 + H_2O + energy

Where the intermediates are butyric acid, mercaptans and hydrogen sulfide gas. At least two general forms of bacteria act in balance in a wastewater digester: Saprophytic organisms and Methane Fermenters. The saprophytes exist on dead or decaying materials. The methane fermenters live on the volatile acids produced by these saprophytes. The methane fermenting bacteria require a pH range of 6.6 to 7.6 to be able to live and reproduce. Aerobic conditions indicate that dissolved oxygen is present. Aerobic bacteria require oxygen to live and thrive. When aerobes decompose organics in the water, the result is carbon dioxide and water.

Aerobic:

Organics + Oxygen-→ CO_2 + H_2O + energy

Dissolved Oxygen in a water sample can be detrimental to metal pipes in high concentrations because oxygen helps accelerate corrosion. Oxygen is an important component in water plant operations. Its primary value is to oxidize iron and manganese into forms that will precipitate out of the water. It also removes excess carbon dioxide. The amount of dissolved oxygen in a water sample will affect the taste of drinking water also.

Methods of Determination

There are two methods that we will be using in the lab. The membrane electrode method procedure is based on the rate of diffusion of molecular oxygen across a membrane. The other is a titrimetric procedure (Winkler Method) based on the oxidizing property of the (DO). Many factors determine the solubility of oxygen in a water sample. Temperature, atmospheric pressure, salinity, biological activity and pH all have an effect on the (DO) content.

Iodometric Test

The iodometric (titration) test is very precise and reliable for (DO) analysis of samples free from particulate matter, color and chemical interferences. Reactions take place with the addition of certain chemicals that liberate iodine equivalent to the original (DO) content. The iodine is then measured to the starch iodine endpoint. We then calculate the dissolved oxygen from how much titrate we use. Certain oxidizing agents can liberate iodine from iodides (positive interference), and some reducing agents reduce iodine to iodide (negative interferences). The alkaline Iodide-Azide reagent effectively removes interference caused by nitrates in the water sample, so a more accurate determination of (DO) can be made.

Methods of analysis are highly dependent on the source and characteristics of the sample. The membrane electrode method involves an oxygen permeable plastic membrane that serves as a diffusion barrier against impurities, Only molecular oxygen passes through the membrane and is measured by the meter. This method is excellent for field testing and continuous monitoring. Membrane electrodes provide an excellent method for (DO) analysis in polluted, highly colored turbid waters and strong waste effluents.

These interferences could cause serious errors in other procedures. Prolonged usage in waters containing such gases as H_2S tends to lower cell sensitivity. Frequent changing and calibrating of the electrode will eliminate this interference.

Samples are taken in BOD bottles where agitation or contact with air is at a minimum. Either condition can cause a change in the gaseous content. Samples must be determined immediately for accurate results. The dissolved oxygen test is the one of the most important analyses in determining the quality of natural waters. The effect of oxidation wastes on streams, the suitability of water for fish and other organisms and the progress of self-purification can all be measured or estimated from the dissolved oxygen content. In aerobic sewage treatment units, the minimum objectionable odor potential, maximum treatment efficiency and stabilization of wastewater are dependent on maintenance of adequate dissolved oxygen. Frequent dissolved oxygen measurement is essential for adequate process control.

Terms

Aerobic (AIR-O-bick) a condition in which free or dissolved oxygen is present in the aquatic environment.

Aerobic Bacteria (aerobes)– bacteria which will live and reproduce only in an environment containing oxygen. Oxygen combined chemically, such as in water molecules (H_2O), cannot be used for respiration by aerobes.

Anaerobic (AN-air O-bick)- a condition in which ***"free"*** or dissolved oxygen is not present in the aquatic environment.

Anaerobic Bacteria (anaerobes) – bacteria that thrive without the presence of oxygen.

Saprophytic bacteria – bacteria that break down complex solids to volatile acids.

Methane Fermenters – bacteria that break down the volatile acids to methane (CH_4) carbon dioxide (CO_2) and water (H_2O).

Oxidation – the addition of oxygen to an element or compound, or removal of hydrogen or an electron from an element or compound in a chemical reaction. The opposite of reduction.

Reduction – the addition of hydrogen, removal of oxygen or addition of electrons to an element or compound. Under anaerobic conditions in wastewater, sulfur or compounds elemental sulfur are reduced to H_2S or sulfide ions.

Procedure for Dissolved Oxygen Determination

METER-PROBE METHOD

1. Collect a water sample in the clean 300-ml glass stoppered BOD bottle for two or three minutes to make sure there are no air bubbles trapped in the bottle. Do one Tap water sample and one DI water sample. Mark the BOD bottles.

2. Insert the DO probe from the meter into your BOD bottles. Record the DO for Tap and DI water. Now continue with the Winkler Burette method.

PROCEDURES FOR WINKLER BURET METHOD

1. Add the contents of one **MANGANESE SULFATE** powder pillow and one **ALKALINE IODIDE-AZIDE** reagent powder pillow to each of your BOD bottles (TAP and DI)

2. Immediately insert the stoppers so that no air is trapped in the bottles and invert several times to mix. A flocculent precipitate will form. It will be brownish-orange if dissolved oxygen is present or white if oxygen is absent.

3. Allow the samples to stand until the floc has settled and leaves the solution clear (about 10 minutes). Again invert the bottles several times to mix and let stand until the solution is clear.

4. Remove the stoppers and add the contents of one **SULFAMIC ACID** powder pillow to each bottle. Replace the stoppers, being careful not to trap any air bubbles in the bottles, and invert several times to mix. The floc will dissolve and leave a yellow color if dissolved oxygen is present.

5. Measure 200 ml of the prepared solution by filling a clean 250-ml graduated cylinder to the 200-ml mark. Pour the solutions into clean 250-ml Erlenmeyer flasks. Save the last 100 mls for a duplicate.

6. Titrate the prepared solutions with PAO Titrant, 0.025N, to a pale yellow color. Use a white paper under the flask.

7. Add two droppers full of Starch Indicator Solution and swirl to mix. A dark blue color will develop.

8. Continue the titration until the solution changes from dark blue to colorless (end point). Go Slow- drop by drop. Record the burette reading to the nearest 0.01mls.

9. The total number of ml of PAO Titrant used is equal to the mg/L dissolved oxygen.

Dissolved Oxygen

Meter Results

1. De-ionized water _____mg/L

2. Tap water _____mg/L

3. What is the meter procedure measuring?

4. What factors would determine which the best method to use is?

5. What are two forms of bacteria present in a wastewater digester?

Wrinkler Method Results

6. **De-ionized Water**

200ml Sample final Burette reading- initial Burette reading- -_____ = _____mg/L

100ml duplicate final Burette reading- initial Burette reading- -_____dup=_____mg/L
mls x 2

7. **Tap water**

200ml Sample final Burette reading- initial Burette reading- -_____=_____mg/L
mls

100ml Sample final Burette reading initial Burette reading- -_____=_____mg/L
mls x 2

8. What are some factors that can alter the (DO) content prior to testing?

9. Were your samples anaerobic or aerobic?

10. Why is it important to monitor the (DO) content of water and wastewater?

Be specific and give a detailed explanation.

Sludge Volume Index (SVI)

Sludge Volume Index Lab
The Sludge Volume Index (**SVI**) of activated sludge is defined as the volume in milliliters occupied by 1g of activated sludge after settling for 30 minutes. The lower the (**SVI**), the better is the settling quality of the aerated mixed liquor. Likewise, high (**SVI**) of 100 or less is considered a good settling sludge.

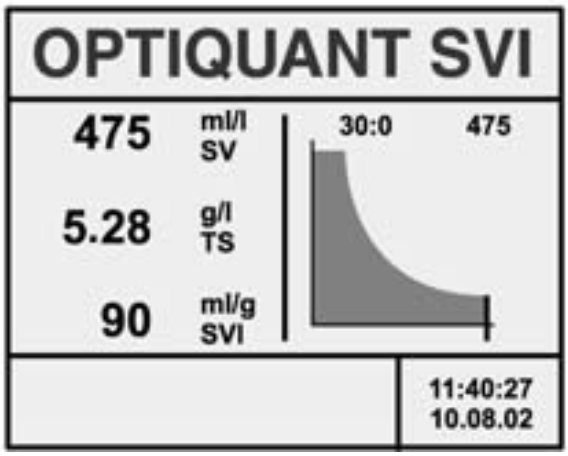
Calculation:
The results obtained from the suspended matter test and settleability test on aerated mixed liquor are used to obtain the SVI.

Calculation:

SVI = ml/L of sludge in settled mixed liquor in 30 min x 1000 mg/g
mg/L of suspended matter in mixed liquor

At last! Automated sludge volume index monitoring
Your wastewater treatment facility relies on timely monitoring of pH, flow, phosphate, ammonia, nitrate, or DO. Now, real-time assessment of sludge conditions with the new OptiQuant SVI™ Sludge Volume and Sludge Volume Index Analyzer complements these key control parameters. Gone are manual samplings and hasty trips to the lab for analysis – it lets operators operate! No more re-mixing, dilutions, or questionable results. The SVI Analyzer's in-situ sampling yields an accurate, representative sample. It automatically detects bulking that signals upset conditions, gives operators better indication of upset root cause and corrective action, and provides on-the-spot response to chemical dosing adjustments. And the SVI Analyzer doesn't make more work for operators, because its unique sampling vessel construction discourages fouling. For complete information contact Hach at WWW.Hach.Com.

Operators select a graphical or numeric SVI controller display. The controller and sampling vessel provide sludge volume monitoring, while an optional OptiQuant™ TS-line suspended solids probe allows automatic calculation of sludge volume index.



Suspended Matter for Mixed Liquor and Return Sludge

Suspended matter in mixed liquor and return sludge can be used to determine process status, estimate the quantity of biomass, and evaluate the results of process adjustments.

Apparatus

- Buchner funnel and adaptor
- Filter flask
- Filter paper 110 mm diam., Whatman 1-4
- 103^0 drying oven
- Desiccator
- Balance
- Graduated Cylinder

Procedure

1. Dry the filter papers in oven at 103^0 c to remove all traces of moisture.

2. Remove papers from oven and desiccate to cool for approximately 5 minutes.

3. Weigh to the nearest 0.01g and record the mass (W_1)

4. Place the paper in the bottom of the Buchner funnel and carefully arrange so that the outer edges lay snugly along the side. Careful not to touch it with your finger. Use a glass rod. Wet the paper, turn on the vacuum and make a good seal, make a pocket covering the bottom of the funnel.

5. Add 20 to 100 mls of sample at a sufficient rate to keep the bottom of the funnel covered, but not fast enough to overflow the pocket made by the filter paper. Record the Volume used.

6. Remove the filter paper with tweezers. Dry in a 103^0 c oven for 30 minutes. Remove and desiccate. Reweigh the filter paper (W_2) to the nearest 0.01g.

Calculation:

mg/L Suspended Matter

$$\frac{(W_2) - (W_1) \times 1000 \text{ ML/L}}{\text{ML Sample}}$$

Where: (W_1) and (W_2) are expressed in mg.
(W_1) = mass of the prepared filter.
(W_2) = mass of the filter and sample after the filtration step.

Settleability Lab

The settled sludge volume of a biological suspension is useful for routine activated sludge plant control. Variations in temperature, sampling and agitation methods, diameter of the settling column, and time between sampling and start of the test can significantly affect results. The same procedure and apparatus should be used each time the test is performed.

Apparatus

-Two settling columns with a minimum volume of 1000 ml.
- A 1000 ml or larger graduated cylinder or Mallory settlometer may be used as a settling column.

Procedure

The settleability test on activated sludge should be run immediately after the sample is taken. The mixed liquor sample should be taken at the effluent end of the aeration tanks, while the return sludge sample should be taken at some point between the final settling tank and the point at which the sludge is mixed with primary effluent.

1. Determine the settle ability of mixed liquor and return sludge by allowing 1000 mls of well mixed samples of each to settle in 1000 ml grad. Cylinder or Mallory settleometer. Care should be taken to minimize floc break up during the transfer of the sample to the cylinder.

2. After 30 minutes, record the volume occupied by the sludge to the nearest 5 ml.

3. The reading at the end of 30 minutes is generally used for plant control. Although the settleability test on return sludge is not used in any of the calculations for activated sludge, the result is helpful in determining whether too much or too little sludge is being returned from the final settling tank.

   Calculation: % Settled Sludge

$$\frac{\text{ml of sludge in settled mixed liquor or return sludge} \times 100}{1000}$$

Sludge Volume Index Lab Report Worksheet

Suspended Mater Calculations:

(W_1) = _____ mg Duplicate (W_1) = _____ mg

(W_2) = _____ mg (W_2) = _____ mg

mls Sample = _____ mls Sample = _____ .

mg/L suspended matter = _____ dup. _____ .

Settleability Calculations:

% settled sludge = _____

$$\frac{\text{(ml of sludge in settled mixed liquor or returned sludge x 100)}}{1000}$$

Sludge Volume Index Calculations:

$$\frac{\text{(ml of sludge in settled mixed liquor in 30 minutes x 1000 mg/g)}}{\text{mg/L of suspended matter in mixed liquor}}$$

Clean Water Act

What is Wastewater Treatment?
Wastewater treatment is the process of cleaning used water and sewage so it can be returned safely to our environment. Wastewater treatment is the last line of defense against water pollution. If you envision the water cycle as a whole, you can clean water produced by wastewater treatment is the same water that eventually ends up back in our lakes and rivers, where we get our drinking water.

Why Are Wastewater Treatment Plants Important?
Wastewater treatment plants are vital to our communities. They protect public health by eliminating disease-causing bacteria from water. By protecting water quality, wastewater treatment plants make it possible for us to safely enjoy the recreational use of clean oceans, lakes, streams and rivers.

33 U.S.C. s/s 1251 et seq. (1977)
The Clean Water Act is a 1977 amendment to the Federal Water Pollution Control Act of 1972, which set the basic structure for regulating discharges of pollutants to waters of the United States.

The law gave the EPA the authority to set effluent standards on an industry basis (technology-based) and continued the requirements to set water quality standards for all contaminants in surface waters. The CWA makes it unlawful for any person to discharge any pollutant from a point source into navigable waters unless a permit (**NPDES**) is obtained under the act.

The 1977 amendments focused on toxic pollutants. In 1987, the PCA was reauthorized and again focused on toxic substances, authorized citizen suit provisions, and funded sewage treatment plants (**POTW's**) under the Construction Grants Program. The CWA provides for the delegation by the EPA of many permitting, administrative, and enforcement aspects of the law to state governments. In states with the authority to implement CWA programs, the EPA still retains oversight responsibilities. In 1972, Congress enacted the first comprehensive national clean water legislation in response to growing public concern for serious and widespread water pollution. The Clean Water Act is the primary federal law that protects our nation’s waters, including lakes, rivers, aquifers, and coastal areas.

Lake Erie was dying. The Potomac River was clogged with blue-green algae blooms that were a nuisance and a threat to public health. Many of the nation's rivers were little more than open sewers and sewage frequently washed up on shore. Fish kills were a common sight. Wetlands were disappearing at a rapid rate.

Today, the quality of our waters has improved dramatically as a result of a cooperative effort by federal, state, tribal and local governments to implement the pollution control programs established in 1972 by the Clean Water Act. The Clean Water Act's primary objective is to restore and maintain the integrity of the nation's waters. This objective translates into two fundamental national goals:

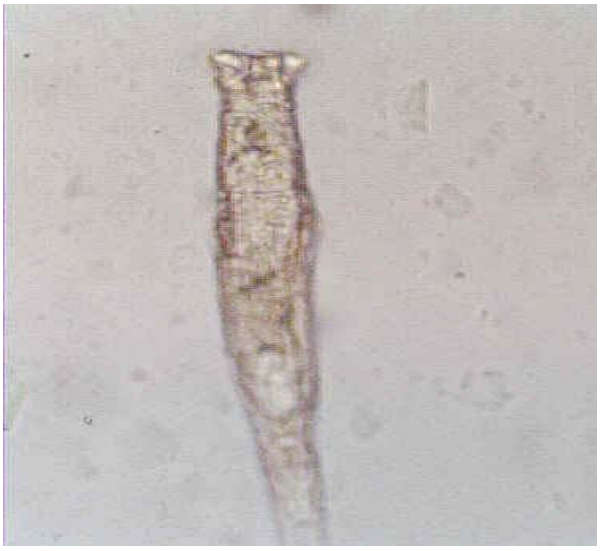
- eliminate the discharge of pollutants into the nation's waters, and
- achieve water quality levels that are fishable and swimmable.

The Clean Water Act focuses on improving the quality of the nation's waters. It provides a comprehensive framework of standards, technical tools and financial assistance to address the many causes of pollution and poor water quality. This includes municipal and industrial wastewater discharges, polluted runoff from urban and rural areas, and habitat destruction. For example, the Clean Water Act requires major industries to meet performance standards to ensure pollution control; charges states, and tribes with setting specific water quality criteria appropriate for their waters and developing pollution control programs to meet them; provides funding to states and communities to help them meet their clean water infrastructure needs; protects valuable wetlands and other aquatic habitats through a permitting process that ensures development, and other activities are conducted in an environmentally sound manner. After 25 years, the act continues to provide a clear path for clean water and a solid foundation for an effective national water program.

In 1972
Only a third of the nation's waters were safe for fishing and swimming. Wetlands losses were estimated at about 460,000 acres annually. Agricultural runoff resulted in the erosion of 2.25 billion tons of soil and the deposit of large amounts of phosphorus and nitrogen into many waters. Sewage treatment plants served only 85 million people.

Today
Two-thirds of the nation's waters are safe for fishing and swimming. The rate of annual wetlands losses is estimated at about 70,000-90,000 acres according to recent studies. The amount of soil lost due to agricultural runoff has been cut by one billion tons annually, and phosphorus and nitrogen levels in water sources are down. Modern wastewater treatment facilities serve 173 million people.

The Future
All Americans will enjoy clean water that is safe for fishing and swimming. We will achieve a net gain of wetlands by preventing additional losses and restoring hundreds of thousands of acres of wetlands. Soil erosion and runoff of phosphorus and nitrogen into watersheds will be minimized, helping to sustain the nation's farming economy and aquatic systems. The nation's waters will be free of effects of sewage discharges.



Rotifer, an excellent MO or Microorganism or Indicator Organism.

Wastewater Treatment WWT Introduction

During the early days of our nation's history, people living in both the cities and the countryside used cesspools and privies to dispose of domestic wastewater. Cities began to install wastewater collection systems in the late nineteenth century because of an increasing awareness of waterborne disease and the popularity of indoor plumbing and flush toilets.

The use of sewage collection systems brought dramatic improvements to public health, further encouraging the growth of metropolitan areas. In the year 2000 approximately 208 million people in the U.S. were served by centralized collection systems.

Wastewater Treatment
In 1892, only 27 American cities provided wastewater treatment. Today, more than 16,000 publicly-owned wastewater treatment plants operate in the United States and its territories. The constructions of wastewater treatment facilities blossomed in the 1920s and again after the passage of the CWA in 1972 with the availability of grant funding and new requirements calling for minimum levels of treatment. Adequate treatment of wastewater, along with the ability to provide a sufficient supply of clean water, has become a major concern for many communities.

What is in Wastewater?
Wastewater is mostly water by weight. Other materials make up only a small portion of wastewater, but can be present in large enough quantities to endanger public health and the environment. Because practically anything that can be flushed down a toilet, drain, or sewer can be found in wastewater, even household sewage contains many potential pollutants. The wastewater components that should be of most concern to homeowners and communities are those that have the potential to cause disease or detrimental environmental effects.

Basic Wastewater Treatment Processes
Physical
Physical processes were some of the earliest methods to remove solids from wastewater, usually by passing wastewater through screens to remove debris and solids. In addition, solids that are heavier than water will settle out from wastewater by gravity. Particles with entrapped air float to the top of water and can also be removed. These physical processes are employed in many modern wastewater treatment facilities today.

Biological
In nature, bacteria and other small organisms in water consume organic matter in sewage, turning it into new bacterial cells, carbon dioxide, and other by-products. The bacteria normally present in water must have oxygen to do their part in breaking down the sewage. In the 1920s, scientists observed that these natural processes could be contained and accelerated in systems to remove organic material from wastewater. With the addition of oxygen to wastewater, masses of microorganisms grew and rapidly metabolized organic pollutants.

Any excess microbiological growth could be removed from the wastewater by physical processes. Activated Sludge is a suspended growth process for removing organic matter from sewage by saturating it with air and microorganisms that can break down the organic matter. Advanced Treatment involves treatment levels beyond secondary treatment.

Chemical
Chemicals can be used to create changes in pollutants that increase the removal of these new forms by physical processes. Simple chemicals such as alum, lime or iron salts can be added to wastewater to cause certain pollutants, such as phosphorus, to floc or bunch together into large, heavier masses which can be removed faster through physical processes. Over the past 30 years, the chemical industry has developed synthetic inert chemicals known as polymers to further improve the physical separation step in wastewater treatment. Polymers are often used at the later stages of treatment to improve the settling of excess microbiological growth or biosolids.

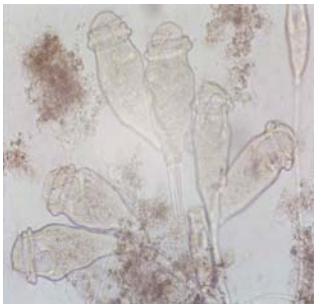
Organisms
Many different types of organisms live in wastewater and some are essential contributors to treatment. A variety of bacteria, protozoa, and worms work to break down certain carbon-based (organic) pollutants in wastewater by consuming them. Through this process, organisms turn wastes into carbon dioxide, water, or new cell growth.

Bacteria and other microorganisms are particularly plentiful in wastewater and accomplish most of the treatment. Most wastewater treatment systems are designed to rely in large part on biological processes.

Pathogens
Many disease-causing viruses, parasites, and bacteria also are present in wastewater and enter from almost anywhere in the community. These pathogens often originate from people and animals are infected with or are carriers of a disease. Graywater and blackwater from typical homes contain enough pathogens to pose a risk to public health. Other likely sources in communities include hospitals, schools, farms, and food processing plants.

Some illnesses from wastewater-related sources are relatively common. Gastroenteritis (shown below) can result from a variety of pathogens in wastewater, and cases of illnesses caused by the parasitic protozoa Giardia lambia and Cryptosporidium are not unusual in the U.S. Other important wastewater-related diseases include hepatitis A, typhoid, polio, cholera, and dysentery. Outbreaks of these diseases can occur as a result of drinking water from wells polluted by wastewater, eating contaminated fish, or recreational activities in polluted waters. Some illnesses can be spread by animals and insects that come in contact with wastewater.

Even municipal drinking water sources are not completely immune to health risks from wastewater pathogens. Drinking water treatment efforts can become overwhelmed when water resources are heavily polluted by wastewater. For this reason, wastewater treatment is as important to public health as drinking water treatment.



Ciliate

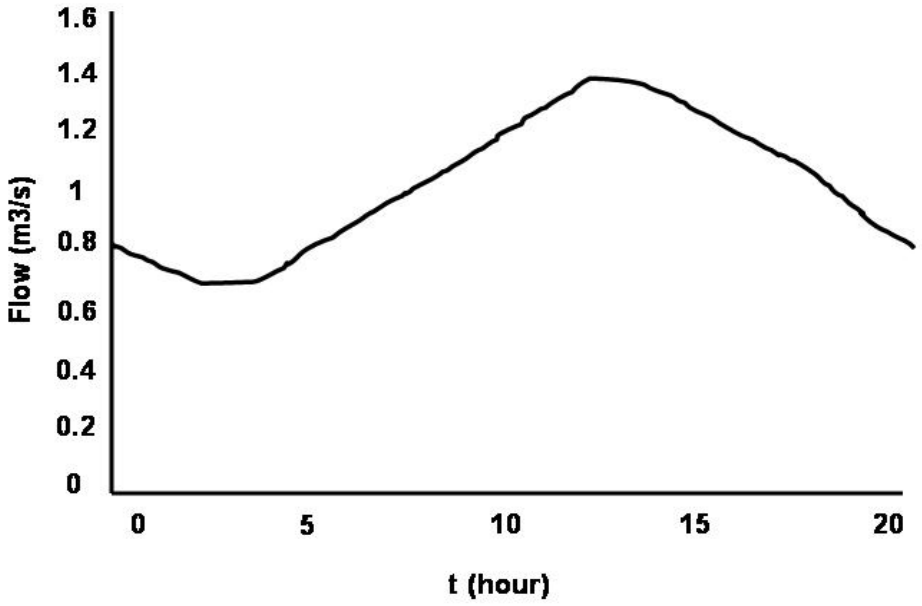
Vorticella is a stalked ciliate. There are at least a dozen species found in activated sludge ranging in length from about 30 to 150 µm. These organisms are oval to round shaped, have a contractile stalk, a domed feeding zone, and a water vacuole located near the terminal end of the feeding cavity.

Domestic Wastewater Characteristics

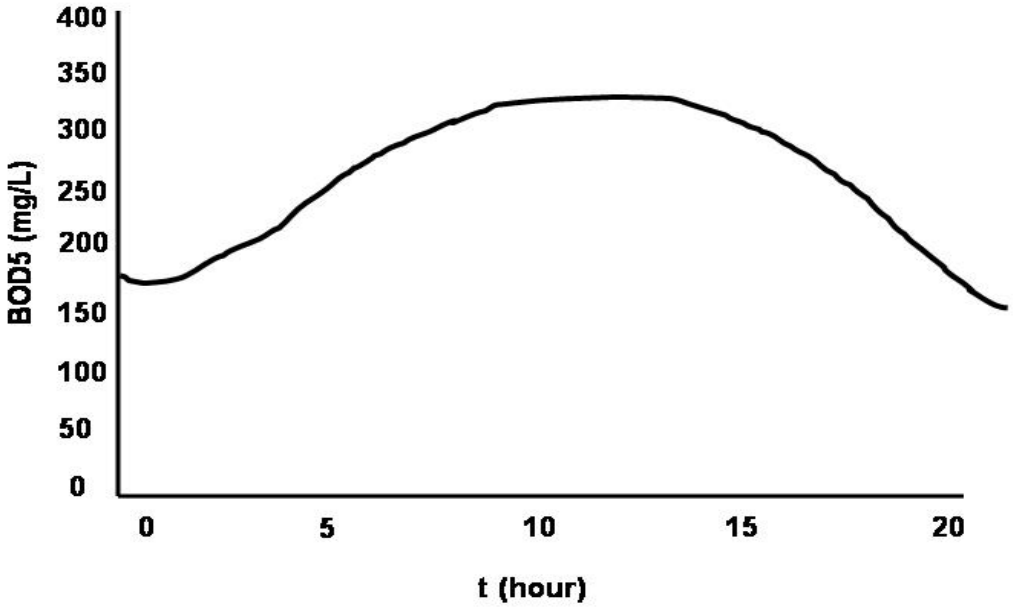
Typical major pollutant characteristics of domestic wastewater

Type	Pollutant	Conc. (mg/L)
Physical	Total Suspended Solids	300
	Volatile Suspended Solids	240
	Fixed Suspended Solids	60
	Total Dissolved Solids	440
	Volatile Suspended Solids	175
	Fixed Suspended Solids	265
	Temperature	10 - 25 ^{o}C
	Color	Grey - Black
Chemical	BOD_5	250
	COD	500
	TOC	160
	Total N	40
	Organic N	15
	Free ammonia N	25
	Nitrite N	0
	Nitrates N	0
	Total P	9
	Organic P	4
	Inorganic P	5
	Alkalinity	100
	Fats, oil and grease (FOG)	100
Microbiological	Total coliform	10^8 - 10^9 MPN/L
	Fecal coliform	10^7 - 10^8 MPN/L
	Non-fecal coliform	$9x10^7$ - $9x10^8$ MPN/L
	Total viruses	1,000-10,0000 infectious units/L

Typical Flow Rate of Domestic Wastewater



Typical BOD5 Variation of Domestic Wastewater



Effects of Wastewater Pollutants

Effect of BOD

- Depletes dissolved oxygen from streams, lakes and oceans.
- May cause death of aerobic organisms (fish kills, etc.).
- Increases anaerobic properties of water.

Effect of TSS

- **Increases turbidity**
  - Less light - reduced photosynthesis.
  - Causes fish's gills to get plugged up.
- **Increases silting**
  - Reduces lifetime of lakes.
  - Changes benthic (i.e., bottom) ecology.

Effects of Phosphorous and Nitrogen

- **Increases algal photosynthesis (eutrophication)**
  - Increased plant life on surface.
  - Reduces light in lower levels.

Additional Effects of Nitrogen

- Organic nitrogen and ammonia are converted to nitrates in water.
- Nitrates are converted to nitrites in digestive system.
- Nitrites are assimilated into blood stream where they are converted by respired oxygen to nitrates.
- May cause suffocation (blue baby syndrome).

Effect of pH

- Organisms are very susceptible to acids and bases.
- Recommended to have near neutral conditions (6.5 - 8.5).

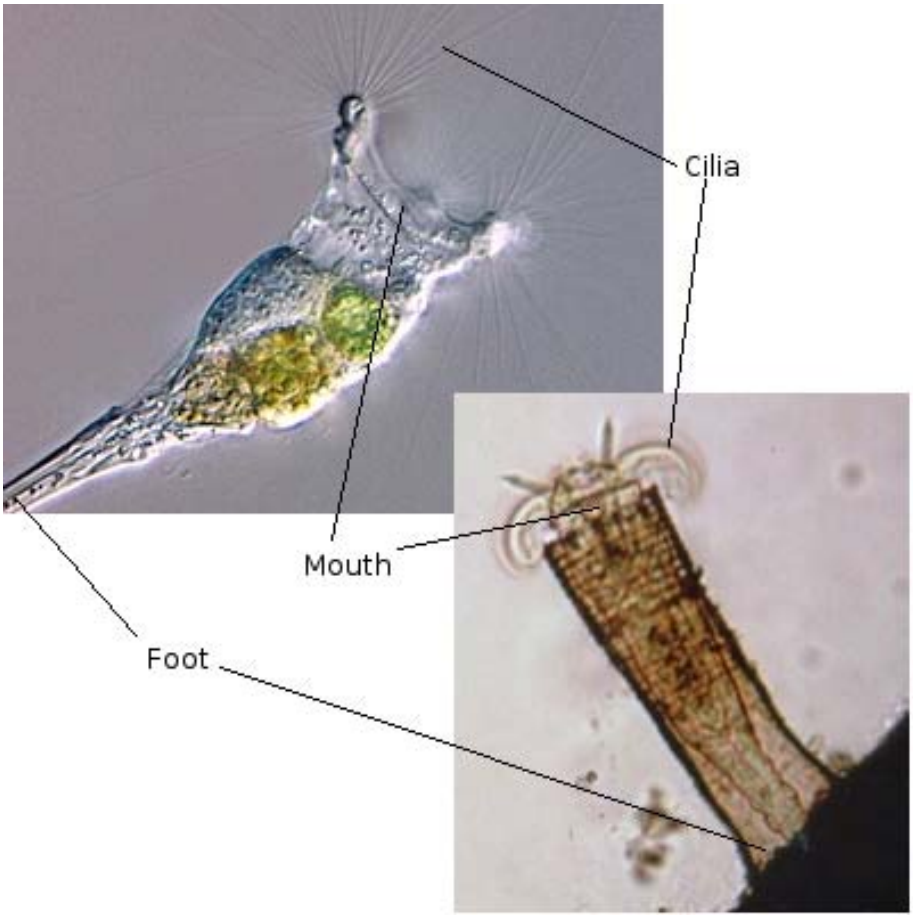
Effect of Pathogens
May infect:

- Humans
- Animals

Domestic waste overflow at the head works. Yes, incredibly headworks do over-flow, usually due to rags, grease and debris or operator error. We do not like to see this happening and are very careful about letting the public and state regulatory agencies see this activity. One activity the State does not want to see but will happen during a rain storm is bypassing untreated waste to the outfall.

Vortecella

Ciliate



Rotifer

Organic Matter

Organic materials are found everywhere in the environment. They are composed of the carbon-based chemicals that are the building blocks of most living things. Organic materials in wastewater originate from plants, animals, or synthetic organic compounds, and enter wastewater in human wastes, paper products, detergents, cosmetics, foods, and from agricultural, commercial, and industrial sources.

Organic compounds normally are some combination of carbon, hydrogen, oxygen, nitrogen, and other elements. Many organics are proteins, carbohydrates, or fats and are biodegradable, which means they can be consumed and broken down by organisms. However, even biodegradable materials can cause pollution. In fact, too much organic matter in wastewater can be devastating to receiving waters.

Large amounts of biodegradable materials are dangerous to lakes, streams, and oceans, because organisms use dissolved oxygen in the water to break down the wastes. This can reduce or deplete the supply of oxygen in the water needed by aquatic life, resulting in fish kills, odors, and overall degradation of water quality. The amount of oxygen organisms need to break down wastes in wastewater is referred to as the biochemical oxygen demand (**BOD**) and is one of the measurements used to assess overall wastewater strength.

Some organic compounds are more stable than others and cannot be quickly broken down by organisms, posing an additional challenge for treatment. This is true of many synthetic organic compounds developed for agriculture and industry.

In addition, certain synthetic organics are highly toxic. Pesticides and herbicides are toxic to humans, fish, and aquatic plants and often are disposed of improperly in drains or carried in stormwater. In receiving waters, they kill or contaminate fish, making them unfit to eat. They also can damage processes in treatment plants. Benzene and toluene are two toxic organic compounds found in some solvents, pesticides, and other products. New synthetic organic compounds are being developed all the time, which can complicate treatment efforts.

Oil and Grease

Fatty organic materials from animals, vegetables, and petroleum also are not quickly broken down by bacteria and can cause pollution in receiving environments. When large amounts of oils and greases are discharged to receiving waters from community systems, they increase BOD and they may float to the surface and harden, causing aesthetically unpleasing conditions. They also can trap trash, plants, and other materials, causing foul odors, attracting flies and mosquitoes and other disease vectors. In some cases, too much oil and grease causes septic conditions in ponds and lakes by preventing oxygen from the atmosphere from reaching the water.

Onsite systems also can be harmed by too much oil and grease, which can clog onsite system drainfield pipes and soils, adding to the risk of system failure. Excessive grease also adds to the septic tank scum layer, causing more frequent tank pumping to be required. Both possibilities can result in significant costs to homeowners.

Petroleum-based waste oils used for motors and industry are considered hazardous waste and should be collected and disposed of separately from wastewater.

Inorganics
Inorganic minerals, metals, and compounds, such as sodium, potassium, calcium, magnesium, cadmium, copper, lead, nickel, and zinc are common in wastewater from both residential and nonresidential sources. They can originate from a variety of sources in the community including industrial and commercial sources, stormwater, and inflow and infiltration from cracked pipes and leaky manhole covers. Most inorganic substances are relatively stable, and cannot be broken down easily by organisms in wastewater.

Large amounts of many inorganic substances can contaminate soil and water. Some are toxic to animals and humans and may accumulate in the environment. For this reason, extra treatment steps are often required to remove inorganic materials from industrial wastewater sources. For example, heavy metals which are discharged with many types of industrial wastewaters are difficult to remove by conventional treatment methods. Although acute poisonings from heavy metals in drinking water are rare in the U.S., potential long-term health effects of ingesting small amounts of some inorganic substances over an extended period of time are possible.

Nutrients
Wastewater often contains large amounts of the nutrients nitrogen and phosphorus in the form of nitrate and phosphate, which promote plant growth. Organisms only require small amounts of nutrients in biological treatment, so there normally is an excess available in treated wastewater. In severe cases, excessive nutrients in receiving waters cause algae and other plants to grow quickly depleting oxygen in the water, deprived of oxygen, fish and other aquatic life die, emitting foul odors. Nutrients from wastewater have also been linked to ocean "red tides" that poison fish and cause illness in humans. Nitrogen in drinking water may contribute to miscarriages and is the cause of a serious illness in infants called methemoglobinemia or "blue baby syndrome."

Solids
Solid materials in wastewater can consist of organic and/or inorganic materials and organisms. The solids must be significantly reduced by treatment or they can increase BOD when discharged to receiving waters and provide places for microorganisms to escape disinfection. They also can clog soil absorption fields in onsite systems.

Settleable solids: Certain substances, such as sand, grit, and heavier organic and inorganic materials settle out from the rest of the wastewater stream during the preliminary stages of treatment. On the bottom of settling tanks and ponds, organic material makes up a biologically active layer of sludge that aids in treatment.

Suspended solids: Materials that resist settling may remain suspended in wastewater. Suspended solids in wastewater must be treated, or they will clog soil absorption systems or reduce the effectiveness of disinfection systems.

Dissolved solids: Small particles of certain wastewater materials can dissolve, like salt in water. Some dissolved materials are consumed by microorganisms in wastewater, but others, such as heavy metals, are difficult to remove by conventional treatment. Excessive amounts of dissolved solids in wastewater can have adverse effects on the environment.

Gases
Certain gases in wastewater can cause odors, affect treatment, or are potentially dangerous. Methane gas, for example, is a byproduct of anaerobic biological treatment and is highly combustible. Special precautions need to be taken near septic tanks, manholes, treatment plants, and other areas where wastewater gases can collect.

Mechanical Bar Screens. Operators are necessary to pick up trash that is blown off the rakes.

Here is a grinder pump that is installed after the bar screens. This debris is sent to the landfill.

Caked grease stuck on weir.

Floating scum in primary clarifier.

Maintenance on a circular clarifier should be performed annually.

Scum Box

Scraping mechanism inside a clarifier.

Scum rake collecting oil and other floating particles.

Hydrogen Sulfide and Ammonia

The gases hydrogen sulfide and ammonia can be toxic and pose asphyxiation hazards. Ammonia as a dissolved gas in wastewater also is dangerous to fish. Both gases emit odors, which can be a serious nuisance. Unless effectively contained or minimized by design and location, wastewater odors can affect the mental well-being and quality of life of residents. In some cases, odors can even lower property values and affect the local economy.

Hydrogen sulfide or H_2S problems are very common in the collection and wastewater system. There are many chemicals used to help or treat this problem. Here are a few used in the treatment of hydrogen sulfide problems: Salts of zinc, lime, hydrogen peroxide, chlorine and magnesium hydroxide. Hydrogen sulfide production in collection systems can cause a number of problems such as corrosion of the pipes, manholes, and creation of hazardous atmospheres and foul odors. The best method of controlling hydrogen sulfide is to eliminate its habitat or growth area by keeping sewers cleaner, this will harbor fewer slime bacteria. Here are some important statements regarding the reduction of hydrogen sulfide: Salts of zinc and iron may precipitate sulfides, lime treatments can also kill bacteria which produce hydrogen sulfide, but this creates a sludge disposal problem and chlorination is effective at reducing the bacteria which produce hydrogen sulfide. Hydrogen sulfide conditions occur in the sewer system because of the lack of oxygen.

Pollutants, Oxygen-Demanding Substances
Dissolved oxygen is a key element in water quality that is necessary to support aquatic life. A demand is placed on the natural supply of dissolved oxygen by many pollutants in wastewater. This is called biochemical oxygen demand, or BOD, and is used to measure how well a sewage treatment plant is working. If the effluent, the treated wastewater produced by a treatment plant, has a high content of organic pollutants or ammonia, it will demand more oxygen from the water and leave the water with less oxygen to support fish and other aquatic life. Organic matter and ammonia are “oxygen-demanding” substances. Oxygen-demanding substances are contributed by domestic sewage and agricultural and industrial wastes of both plant and animal origin, such as those from food processing, paper mills, tanning, and other manufacturing processes. These substances are usually destroyed or converted to other compounds by bacteria if there is sufficient oxygen present in the water, but the dissolved oxygen needed to sustain fish life is used up in this break down process.

Pathogens
Disinfection of wastewater and chlorination of drinking water supplies has reduced the occurrence of waterborne diseases such as typhoid fever, cholera, and dysentery, which remain problems in underdeveloped countries while they have been virtually eliminated in the infectious micro-organisms, or pathogens, may be carried into surface and groundwater by sewage from cities and institutions, by certain kinds of industrial wastes, such as tanning and meat packing plants, and by the contamination of storm runoff with animal wastes from pets, livestock and wild animals, such as geese or deer. Humans may come in contact with these pathogens either by drinking contaminated water or through swimming, fishing, or other contact activities. Modern disinfection techniques have greatly reduced the danger of waterborne disease.

Nutrients
Carbon, nitrogen, and phosphorus are essential to living organisms and are the chief nutrients present in natural water. Large amounts of these nutrients are also present in sewage, certain industrial wastes, and drainage from fertilized land. Conventional secondary biological treatment processes do not remove the phosphorus and nitrogen to any substantial extent. They may convert the organic forms of these substances into mineral form, making them more usable by plant life. When an excess of these nutrients over-stimulates the growth of water plants, the result causes unsightly conditions, interferes with drinking water treatment processes, and causes unpleasant and disagreeable tastes and odors in drinking water.

The release of large amounts of nutrients, primarily phosphorus but occasionally nitrogen, causes nutrient enrichment which results in excessive growth of algae. Uncontrolled algae growth blocks out sunlight and chokes aquatic plants and animals by depleting dissolved oxygen in the water at night. The release of nutrients in quantities that exceed the affected waterbody's ability to assimilate them results in a condition called eutrophication or cultural enrichment.

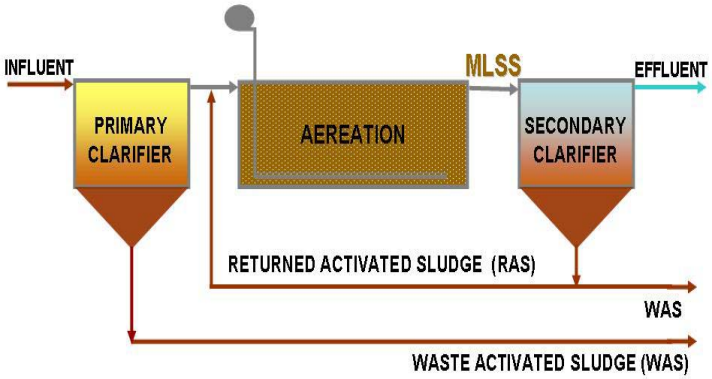
Inorganic and Synthetic Organic Chemicals
A vast array of chemicals is included in this category. Examples include detergents, household cleaning aids, heavy metals, pharmaceuticals, synthetic organic pesticides and herbicides, industrial chemicals, and the wastes from their manufacture. Many of these substances are toxic to fish and aquatic life and many are harmful to humans. Some are known to be highly poisonous at very low concentrations. Others can cause taste and odor problems, and many are not effectively removed by conventional wastewater treatment.

Thermal
Heat reduces the capacity of water to retain oxygen. In some areas, water used for cooling is discharged to streams at elevated temperatures from power plants and industries. Even discharges from wastewater treatment plants and storm water retention ponds affected by summer heat can be released at temperatures above that of the receiving water, and elevate the stream temperature. Unchecked discharges of waste heat can seriously alter the ecology of a lake, a stream, or estuary.

Primary Treatment
The initial stage in the treatment of domestic wastewater is known as primary treatment. Coarse solids are removed from the wastewater in the primary stage of treatment. In some treatment plants, primary and secondary stages may be combined into one basic operation. At many wastewater treatment facilities, influent passes through preliminary treatment units before primary and secondary treatment begins. One of the most common forms of pollution control in the United States is ***wastewater treatment***. The country has a vast system of collection sewers, pumping stations, and treatment plants. Sewers collect the wastewater from homes, businesses, and many industries, and deliver it to plants for treatment. Most treatment plants were built to clean wastewater for discharge into streams or other receiving waters, or for reuse.

Years ago, when sewage was dumped into waterways, a natural process of purification began. First, the sheer volume of clean water in the stream diluted wastes. Bacteria and other small organisms in the water consumed the sewage and other organic matter, turning it into new bacterial cells; carbon dioxide and other products. Today's higher populations and greater volume of domestic and industrial wastewater require that communities give nature a helping hand. The basic function of wastewater treatment is to speed up the natural processes by which water is purified.

There are **two basic stages** in the treatment of wastes, ***primary*** and ***secondary***. In the primary stage, solids are allowed to settle and removed from wastewater. The secondary stage uses biological processes to further purify wastewater. Sometimes, these stages are combined into one operation.



Preliminary Treatment

The Preliminary Treatment is purely physical stage consisting of Coarse Screening, Raw Influent Pumping, Static Fine Screening, Grit Removal, and Selector Tanks. The raw wastewater enters from the collection system into the Coarse Screening process. After the wastewater has been screened, it may flow into a grit chamber where sand, grit, cinders, and small stones settle to the bottom.

Removing the grit and gravel that washes off streets or land during storms is very important, especially in cities with combined sewer systems. Large amounts of grit and sand entering a treatment plant can cause serious operating problems, such as excessive wear of pumps and other equipment, clogging of aeration devices, or taking up capacity in tanks that is needed for treatment.

In some plants, another finer screen is placed after the grit chamber to remove any additional material that might damage equipment or interfere with later processes. The grit and screenings removed by these processes must be periodically collected and trucked to a landfill for disposal or are incinerated.

Collected Grit

The Coarse Screening consists of a basket shaped bar screen which collects larger debris (several inches in diameter) prior to the Raw Influent Pumping. This debris is removed and placed into a dumpster for disposal into the landfill.

The wastewater then passes into the Raw Influent Pumping process that consists of submersible centrifugal pumps. These influent pumps operate under a principal termed prerotation, which allows them to vary their pump rate hydraulically without the use of complex and expensive electronics.

Manual and Mechanical Bar Screens

The flow then passes into the Static Fine Screening process which consists of two stationary (or static) screens which remove finer debris not captured by the coarse screens. This screened debris is then dewatered and collected in hoppers for disposal into a landfill.

The wastewater then passes into the Grit Removal process which consists of two vortex grit separators which produce a whirlpool action to force the finest debris to the outside perimeter for subsequent collection. This debris is then collected in hoppers, dewatered, and disposed into a landfill. The screened and de-gritted wastewater then enters into Primary Sedimentation.

Fine Screening

Primary Sedimentation
With the screening completed and the grit removed, wastewater still contains dissolved organic and inorganic constituents along with suspended solids. The suspended solids consist of minute particles of matter that can be removed from the wastewater with further treatment such as sedimentation or gravity settling, chemical coagulation, or filtration.

Primary Clarifier

Pollutants that are dissolved or are very fine and remain suspended in the wastewater are not removed effectively by gravity settling. When the wastewater enters a sedimentation tank, it slows down and the suspended solids gradually sink to the bottom. This mass of solids is called primary sludge. Various methods have been devised to remove solids, newer plants have some type of mechanical equipment to remove the settled solids and some plants remove solids continuously while others do so at intervals.

Secondary Treatment
After the wastewater has been through Primary Treatment processes, it flows into the next stage of treatment called secondary. Secondary treatment processes can remove up to 90 percent of the organic matter in wastewater by using biological treatment processes. The two most common conventional methods used to achieve secondary treatment are attached growth processes and suspended growth processes.

The Secondary Treatment stage consists of a biological process such as **Oxidation Ditches** and a physical process, **Secondary Clarification**. The Preliminary Treatment stage removed as much solids as possible using physical processes, however, very fine solids are still present that cannot be removed physically.

The wastewater enters from Preliminary Treatment into the Oxidation Ditches process which is a biological process consisting of two large oval shaped basins which are capable of removing these finer solids. This is accomplished by maintaining a population of microorganisms within the oxidation basins which consume the very fine solids (which are primarily organic) and also adhere to the solids themselves. By consuming and adhering to these finer solids they form larger and heavier aggregates that can by physically separated. Thus, after this process has taken place within the Oxidation Ditches the wastewater then enters Secondary Clarification process which can provide this physical separation.

Nitrogen and Phosphorus Removal Technologies

Introduction
This section provides information on a number of different technologies that can reduce nitrogen and phosphorus levels. The actual technology selected will be site-specific and dependent on many factors including soil conditions, influent water quality, required effluent levels, disposal options, availability of land, cost, etc. In some cases, a combination of technologies may be necessary to effectively remove all the contaminants of concern. Small system owners and operators should work closely with their state onsite and decentralized program staff as well as engineers to ensure that the technologies selected will work effectively in combination to achieve the effluent goals.

Nutrient Removal Technologies
Fixed-film systems - Aerobic/anaerobic trickling filter package plant
Fixed-film systems (FFSs) are biological treatment processes that employ a medium such as rock, plastic, wood, or other natural or synthetic solid material that will support biomass on its surface and within its porous structure (USEPA, 2008c). Trickling filter FFSs are typically constructed as beds of media through which wastewater flows. Oxygen is normally provided by natural or forced ventilation. Commercial on-site systems use synthetic media and receive wastewater from overlying sprayheads for aerobic treatment and nitrification. Nitrified effluent returns to the anoxic zone to mix with either septic tank contents or incoming septic tank effluent for denitrification. A portion of the denitrified effluent is discharged for disposal or further treatment. Aerobic tanks are available in residential or small community sizes. Typical trickling filters systems currently available are capable of producing effluent BOD and TSS concentrations of 5 to 40 mg/L. Nitrogen removal typically varies from 0 to 35 percent although removal percentages as high as 65% have been demonstrated through USEPA's Environmental Technology Verification (ETV) program. Phosphorus removal is typically 10 to 15 percent.

Higher removal occurs at low loading rates in warm climates. Systems can be configured for single-pass use where the treated water is applied to the trickling filter once before being disposed of, or for multi-pass use where a portion of the treated water is cycled back to the septic tank and re-treated via a closed loop.

Multi-pass systems result in higher treatment quality and assist in removing Total Nitrogen (TN) levels by promoting nitrification in the aerobic media bed and denitrification in the anaerobic septic tank. Factors affecting performance include influent wastewater characteristics, hydraulic and organic loading, medium type, maintenance of optimal DO levels, and recirculation rates.

Sequencing batch reactor (SBR)
The SBR process is a sequential suspended growth (activated sludge) process in which all major steps occur in the same tank in sequential order (USEPA, 2008d). The SBR system is typically found in packaged configurations for onsite and small community or cluster applications. The major components of the package include the batch tank, aerator, mixer, decanter device, process control system (including timers), pumps, piping, and appurtenances. Aeration may be provided by diffused air or mechanical devices. SBRs are often sized to provide mixing as well and are operated by the process control timers.

Mechanical aerators have the added value of potential operation as mixers or aerators. The decanter is a critical element in the process. Several decanter configurations are available, including fixed and floating units. At least one commercial package employs a thermal processing step for the excess sludge produced and wasted during the "idle" step. The key to the SBR process is the control system, which consists of a combination of level sensors, timers, and microprocessors which can be configured to meet the needs of the system.

SBRs can be designed and operated to enhance removal of nitrogen, phosphorus, and ammonia, in addition to removing TSS and BOD. Package plant SBRs are suitable for areas with little land, stringent treatment requirements, and small wastewater flows such as RV parks or mobile homes, campgrounds, construction sites, rural schools, hotels, and other small applications. These systems are also useful for treating pharmaceutical, brewery, dairy, pulp and paper, and chemical wastes (USEPA, 2000d).

Intermittent sand filters (ISF)
ISF is used to describe a variety of packed-bed filters of sand or other granular materials available on the market (USEPA, 2008g). Sand filters provide advanced secondary treatment of settled wastewater or septic tank effluent. They consist of a lined (e.g., impervious PVC liner on sand bedding) excavation or structure filled with uniform washed sand that is placed over an underdrain system. The wastewater is directed onto the surface of the sand through a distribution network and allowed to percolate through the sand to the underdrain system. The underdrain system collects the filter effluent for further processing or discharge.

Sand filters are aerobic, fixed-film bioreactors. Bioslimes from the growth of microorganisms develop as films on the sand particle surfaces. The microorganisms in the slimes capture soluble and colloidal waste materials in the wastewater as it percolates over the sand surfaces. The captured materials are metabolized into new cell mass or degraded under aerobic conditions to carbon dioxide and water. Most biochemical treatment occurs within approximately 6 inches of the filter surface. Other treatment mechanisms that occur in sand filters include physical processes, such as straining and sedimentation, to remove suspended solids within the pores of the media. Most suspended solids are strained out at the filter surface.

Chemical adsorption can occur throughout the media bed. Adsorption sites in the media are usually limited, however. The capacity of the media to retain ions depends on the target constituent, the pH, and the mineralogy of the media. Phosphorous is one element of concern in wastewater that can be removed in this manner, but the number of available adsorption sites is limited by the characteristics of the media.

Sand filters can be used for a broad range of applications, including single-family residences, large commercial establishments, and small communities. Sand filters are frequently used to pretreat septic tank effluent prior to subsurface infiltration onsite where the soil has insufficient unsaturated depth above ground water or bedrock to achieve adequate treatment. They are also used to meet water quality requirements (with the possible exception of fecal coliform removal) before direct discharge to surface water. Sand filters are used primarily to treat domestic wastewater, but they have been used successfully in treatment trains to treat wastewaters high in organic materials such as those from restaurants and supermarkets. Single-pass ISF filters are most frequently used for smaller applications and sites where nitrogen removal is not required. However, they can be combined with anoxic processes to significantly increase nitrogen removal.

Recirculating sand filters (RSF)
Recirculating filters using sand, gravel, or other media provide advanced secondary treatment of settled wastewater or septic tank effluent (USEPA, 2008h). They consist of a lined (e.g., impervious PVC liner on sand bedding) excavation or structure filled with uniform washed sand that is placed over an underdrain system. The wastewater is directed onto the surface of the sand through a distribution network and allowed to percolate through the sand to the underdrain system. The underdrain system collects and recycles the filter effluent to the recirculation tank for further processing or discharge.

The basic components of recirculating filters include a recirculation/dosing tank, pump and controls, distribution network, filter bed with an underdrain system, and a return line. The return line or the underdrain must split the flow to recycle a portion of the filtrate to the recirculation/dosing tank. A small volume of wastewater and filtrate is dosed to the filter surface on a timed cycle 1 to 3 times per hour.

Recirculation ratios are typically between 3:1 and 5:1. In the recirculation tank, the returned aerobic filtrate mixes with the anaerobic septic tank effluent before being reapplied to the filter. RSFs can be used for a broad range of applications, including single-family residences, large commercial establishments, and small communities. They produce a high quality effluent with approximately 85 to 95 percent BOD and TSS removal. In addition, almost complete nitrification is achieved.

Denitrification also has been shown to occur in RSFs. Depending on modifications in design and operation, 50 percent or more of applied nitrogen can be removed (USEPA, 1999). To enhance this capability, they can be combined with a greater supply of biodegradable organic carbon, time, and mixing than is normally available from the conventional recirculation tank.

Natural Systems

The natural systems described here include constructed wetlands and floating aquatic plant treatment systems. Wetland systems are typically described in terms of the position of the water surface and/or the type of vegetation grown. Most natural wetlands are free water surface (FWS) systems where the water surface is exposed to the atmosphere; these include bogs (primary vegetation mosses), swamps (primary vegetation trees), and marshes (primary vegetation grasses and emergent macrophytes) (USEPA, 2000e). subsurface flow (SF) wetlands are specifically designed to treat or polish wastewater and are typically constructed as a bed or channel containing appropriate media.

Constructed wetlands treat wastewater by bacterial decomposition, settling, and filtering. As in tank designs, bacteria break down organic matter in the wastewater, aerobically, anoxically and anaerobically. Oxygen for aerobic decomposition is supplied by the plants growing in the wetland. Solids are filtered and finally settle out of the wastewater within the wetland. After about two weeks in the wetland, effluent is usually discharged by gravity to an unlined wetland bed. If these systems discharge effluent to surface ditches, they require a NPDES permit.

The submerged plant roots do provide substrate for microbial processes. However, the amount of oxygen that emergent macrophytes can transmit from the leaves to their roots is negligible compared to the oxygen demand of wastewater. Therefore subsurface flow wetlands are devoid of oxygen. The lack of oxygen in these subsurface flow systems means that ammonia oxidation via biological nitrification will not occur without the use of an additional unit process, such as a gravel trickling filter for nitrification of the wastewater ammonia. Vertical flow wetland beds are a modification of subsurface flow wetlands which contain gravel or coarse sand and are loaded intermittently at the top surface. Unlike ammonia oxidation, nitrate removal in a subsurface flow wetland can be rapid and effective because the anoxic conditions and carbon sources necessary to support the treatment reactions occur naturally in these systems.

FWS wetlands with long detention times can remove minor amounts of phosphorus through plant uptake, adsorption, complexation, and precipitation. However, removal via plant uptake is limited to phosphorus retained in plant litter that is buried by sediments before plant decomposition occurs (i.e. peat building process). Phosphorus removal is typically greater in the first year or two because of soil absorption and rapidly expanding vegetation but decreases when the system reaches equilibrium, and unburied plant litter releases phosphorus back into the water as it decomposes. Phosphorus removal is also possible with the use of an addition process, such as chemical addition and mixing prior to a final deep settling pond.

Aquatic systems using duckweed have been used for a number of years to treat wastewater for various purposes (WEF, 2001). Duckweed (*Lemna spp.*) are floating macrophytes. Duckweed fronds can double their mass in two days under ideal conditions of nutrient availability, sunlight, and temperature. Although duckweed can be found in most regions, the rate of growth is optimal at 20 to 30^{o} C and they grow best in a pH range of 3.5 to 8.5.

Duckweed can grow about six months per year in most U.S. climates. High levels of BOD and TSS removal have been observed from duckweed systems. To achieve secondary treatment most duckweed systems are coupled with either facultative or aerated ponds. Nitrogen is removed by plant uptake and harvesting, by denitrification, or a combination of the two. Typically less than 1 mg/L of phosphorus can be removed by plant uptake and harvest. If significant phosphorus removal is required, chemical precipitation with alum, ferric chloride, or other chemicals used in a separate treatment step is necessary. The major disadvantage of duckweed systems is the large amount of biomass produced by the rapidly growing plants, which creates a solids handling requirement similar to handling sludge at an aerobic wastewater treatment facility.

Proprietary Filters/Improved and Emerging Technologies
A number of companies have developed proprietary nitrogen and phosphorus removal technologies that can be used at centralized wastewater treatment facilities as well as at onsite, decentralized systems. This section provides a general description of some of these technologies without mentioning specific trade names.

Sustainable Nutrient Recovery
While the U.S. is primarily addressing nutrient removal concerns through development of WQSs and treatment at centralized wastewater facilities, a number of European countries including Switzerland, Sweden, and the Netherlands are conducting research on innovative sustainable nutrient recovery systems. The concept behind these new technologies is to separate and treat toilet waste before it leaves the home or building and mixes with the larger waste stream to be carried to WWTPs.

Recent studies have shown that about 80 percent of the nitrogen and 50 percent of the phosphorus in wastewater are derived from urine although urine makes up only 1 percent of the volume of wastewater (Larsen and Leinert, 2007). Separating the urine from wastewater could offer various advantages: WWTPs could be built on a smaller scale, water bodies will be better protected from nitrogen and phosphorus pollution, nutrients could be recycled for agricultural use, and various constituents of concern including hormones and pharmaceutical compounds could be removed before being mixed with wastewater and released to the environment. A major benefit would be reduced energy consumption at WWTPs as a result of reduced treatment requirements for nitrogen. Also, separating 50 to 60 percent of urine could reduce in-plant nitrogen gas discharges and result in fewer impurities in methane captured from sludge digestion.

Organizations such as the Swiss Federal Institute of Aquatic Science and Technology (Eawag) are currently experimenting with the development and application of “NoMix technology” to separate urine from solid waste at the toilet bowl. While similar in size and shape to current toilets, this new technology has two waste pipes – a small front one that collects and diverts urine into a storage tank, and a larger rear waste pipe that operates like a standard toilet. The first of these toilets were installed in two “eco-villages” in Sweden in 1994 and since then have spread to other locations throughout the country and to Denmark, the Netherlands, and Switzerland. The concept is now taking hold in Austria and Germany. While the pollutant-free urine, or “urevit,” can be spray-applied directly onto agricultural fields; in the Netherlands, a company called Grontmij trucks stored urine to a special treatment plant where the phosphate is precipitated out as a mineral called struvite and used as a fertilizer.

Novaquatis, a branch of Eawag is experimenting with extracting nitrogen and potassium from urine that can be sprayed directly onto crops. Eawag is also experimenting with a pilot decentralized basement sewage plant where domestic wastewater is treated in a MBR so it can be reused for flushing the toilets or watering the garden and the sewage sludge is composted. While still experimental, some of these technologies may have practical future applications if widely applicable low-cost solutions can be found for urine transport, or stable and cost-effective technologies can be developed for decentralized treatment.

While studies of consumer attitudes and acceptance appear to be positive, technological improvements are still needed to prevent clogging in pipes, to identify best treatment options that can be applied in practice; and to identify how and where to convert urine to fertilizer.

Sustainability concerns are also driving the wastewater treatment industry to start looking at sludge as a renewable resource. Historically, agricultural use has been the traditional approach for disposal of municipal sludge due to its high nutrient content for fertilizing crops, and its low cost approach. As scientific advances detect smaller and smaller quantities of contaminants (i.e., heavy metals, pathogenic microorganisms, pharmaceuticals, and personal care products), the public, farming organizations, and the food industry are raising concerns about continuing this practice. As noted above, researchers are discovering that valuable products can be generated from sewage treatment byproducts such as energy extracted from anaerobic digestion, construction materials such as bricks, and nutrients such as phosphorus that can be extracted from sludge and used as fertilizer.

In February 2008, the non-profit Global Water Research Coalition, an international water research alliance formed by 12 world-leading research organizations, released a report titled, *State of Science Report: Energy and Resource Recovery from Sludge* (Kalogo and Monteith, 2008). The report focuses on:
• The international situation of energy and resource recovery from sludge
• How the use of different sludge treatment processes affects the possibility of recovering energy and/or materials from the residual sludge
• The influence of market and regulatory drivers on the fate of the sludge end-product
• The feasibility of energy and resource recovery from sludge
• The social, economic, and environmental performance (triple bottom line or TBL assessment) of current alternatives technologies

Four market drivers are identified and discussed including:
- Sustainability and environmental concerns, such as the threat of soil pollution, global warming and resource depletion
- Rising energy costs and the need of more electricity and heat to operate the plants
- Requirements for high quality of resources for industrial applications, such as calcium phosphate for the phosphate industry
- Regulation as a factor stimulating the development of new technologies

In the report, energy recovery technologies are classified into sludge-to-biogas processes, sludge-to-syngas processes, sludge-to-oil processes, and sludge-to-liquid processes. The technologies available for resource recovery discussed in the report include those to recover phosphorus, building materials, nitrogen, and volatile acids. The report, which covers both established as well as emerging technologies, will be used as the basis for development of the coalition's future strategic research plan on energy and recovery from sludge. As a technical resource, it provides a valuable overview of sludge disposal practices in various countries such as the U.S., the Netherlands, the United Kingdom, Germany, Sweden, Japan, and China; and presents a number of treatment processes for resource recovery.

Other groups have looked at recovering phosphorus from the supernatant from anaerobic digestion. Several different processes have been proposed that rely on precipitation of the phosphorus as either struvite or calcium phosphate. Work is underway on projects in Italy, Germany, the Netherlands, and Canada (SCOPE, 2004).

Nutrient Removal for Small Communities and Decentralized Wastewater Treatment Systems

Approximately 25 percent of the U.S. population is served by onsite septic or decentralized systems. Onsite septic systems treat and dispose of effluent on the same property that produces the wastewater, whereas decentralized treatment refers to onsite or cluster systems that are used to treat and dispose of relatively small volumes of wastewater, generally from dwellings and businesses that are located relatively close together. In many cases, wastewater from several homes is pretreated onsite by individual septic tanks before being transported through alternative sewers to an offsite decentralized treatment unit that is relatively simple to operate and maintain.

The remaining 75 percent of the population is served by centralized wastewater treatment facilities, which collect and treat large volumes of wastewater. There is, in fact, a growing movement toward decentralized or clustered wastewater treatment systems to reduce cost, to provide groundwater recharge near the source, and for speed and ease in siting since they are generally located underground. The use of residential cluster development is gaining in popularity across the U.S. as a means to permanently protect open space, preserve agricultural land, and protect wildlife habitat (Mega et al., 1998). As part of these developments, wastewater systems such as community drainfields, irrigation systems, and package plants are being installed to reduce infrastructure investment and minimize adverse environmental impacts. Additional alternatives that include aerobic tanks, sand filters, and constructed wetlands can be used to reduce nutrient pollution; particularly in sensitive coastal areas or over sensitive, unconfined aquifers used for drinking water (Anderson and Gustafson, 1998).

Phosphorus Removal

Few phosphorus removal processes are well developed for onsite wastewater systems application (USEPA, 2008e). The controlled addition of chemicals such as aluminum, iron, and calcium compounds with subsequent flocculation and sedimentation has had only limited success because of inadequate operation and maintenance of mechanical equipment and excessive sludge production. Most notable successes have come with special filter materials that are naturally high in their concentration of the above chemicals, but their service lives are finite. Studies of high-iron sands and high-aluminum muds indicate that 50 to 95 percent of the phosphorus can be removed. However, the life of these systems has yet to be determined, after which the filter media will have to be removed and replaced. Use of supplemental iron powder mixed with natural sands is also being researched. Aside from specialized filter media, the most likely phosphorus-reduction systems are iron-rich intermittent sand filter (ISF) media and SBRs. These are discussed in more detail below.

Nitrogen Removal

Processes that remove 25 to 50 percent of total nitrogen include aerobic biological systems and media filters, especially recirculating filters (USEPA, 2008f). The vast majority of on-site and cluster nitrogen-removal systems employ nitrification and denitrification biological reactions. Most notable of these are recirculating sand filters (RSFs) with enhanced anoxic modifications, SBRs, and an array of aerobic nitrification processes combined with an anoxic/anaerobic process to perform denitrification. Some of the combinations are proprietary. A few recently developed highly instrumented systems that utilize membrane solids separation following biological nitrification and denitrification are capable of removing total nitrogen down to very low concentrations (i.e. 3 – 4 mg/L TN). Nitrogen removal systems generally are located last in the treatment train prior to subsurface wastewater infiltration system (SWIS) disposal or surface water disposal, in which case a disinfection step is typically required. Usually, the minimum total nitrogen standard that can be regularly met is about 10 mg/L. These technologies can be either above ground or below ground.

Secondary Clarification Process

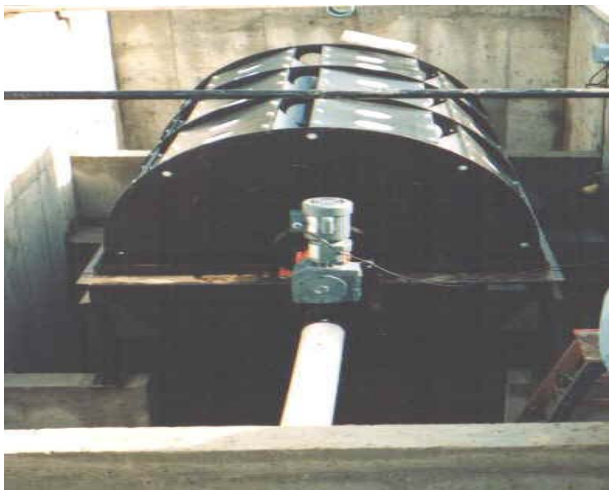
The Secondary Clarification process consists of four rectangular tanks which provide quiescent (or calm) conditions which allow the larger aggregates of solids and microorganisms to settle out for collection. The clear overflow (or upper layer) is collected at the end of the tank and passed onto the Tertiary process for additional treatment if available.

Secondary Clarifier

The majority of microorganism-rich underflow (or lower layer) is re-circulated to Tanks as Return Sludge to help sustain the microorganism population in the Oxidation Ditches process. However, if all the underflow was returned the plant would soon become overloaded with solids, therefore, a small portion of this mixture termed Waste Sludge is removed from the system for disposal. The Waste Sludge is transported into the Solids Handing process for disposal.

Fixed Film Systems

Fixed film systems grow microorganisms on substrates such as rocks, sand or plastic. The wastewater is spread over the substrate, allowing the wastewater to flow past the film of microorganisms fixed to the substrate. As organic matter and nutrients are absorbed from the wastewater, the film of microorganisms grows and thickens. Trickling filters, rotating biological contactors, and sand filters are examples of fixed film systems.



Empty RBC

Suspended Film Systems

Suspended film systems stir and suspend microorganisms in wastewater. As the microorganisms absorb organic matter and nutrients from the wastewater, they grow in size and number. After the microorganisms have been suspended in the wastewater for several hours, they are settled out as sludge. Some of the sludge is pumped back into the incoming wastewater to provide "seed" microorganisms. The remainder is wasted and sent on to a sludge treatment process. Activated sludge, extended aeration, oxidation ditch, and sequential batch reactor systems are all examples of suspended film systems.

Lagoon Systems

Lagoon systems are shallow basins which hold the waste-water for several months to allow for the natural degradation of sewage. These systems take advantage of natural aeration and microorganisms in the wastewater to renovate sewage.

Other Important Wastewater Characteristics
In addition to the many substances found in wastewater, there are other characteristics system designers and operators use to evaluate wastewater. For example, the color, odor, and turbidity of wastewater give clues about the amount and type of pollutants present and treatment necessary. The following are some other important wastewater characteristics that can affect public health and the environment, as well as the design, cost, and effectiveness of treatment.

Sampling Industrial Waste, in this photograph, the Inspector or Sampler is shaking the sample to make sure that the sample is mixed-up before pouring off a smaller sample into the smaller sample bottles on the ground. Normally, these Inspectors or Samplers will work in pairs. Get used to having wastewater and/or industrial waste/odors all over your clothes. But other than that, spiders, grease, confined spaces, irate customers, the interesting odors and dangerous Hydrogen Sulfide gas; this is a good job to have, a secure and well-paying job.

Temperature
The best temperatures for wastewater treatment probably range from 77 to 95 degrees Fahrenheit. In general, biological treatment activity accelerates in warm temperatures and slows in cool temperatures, but extreme hot or cold can stop treatment processes altogether. Therefore, some systems are less effective during cold weather and some may not be appropriate for very cold climates.

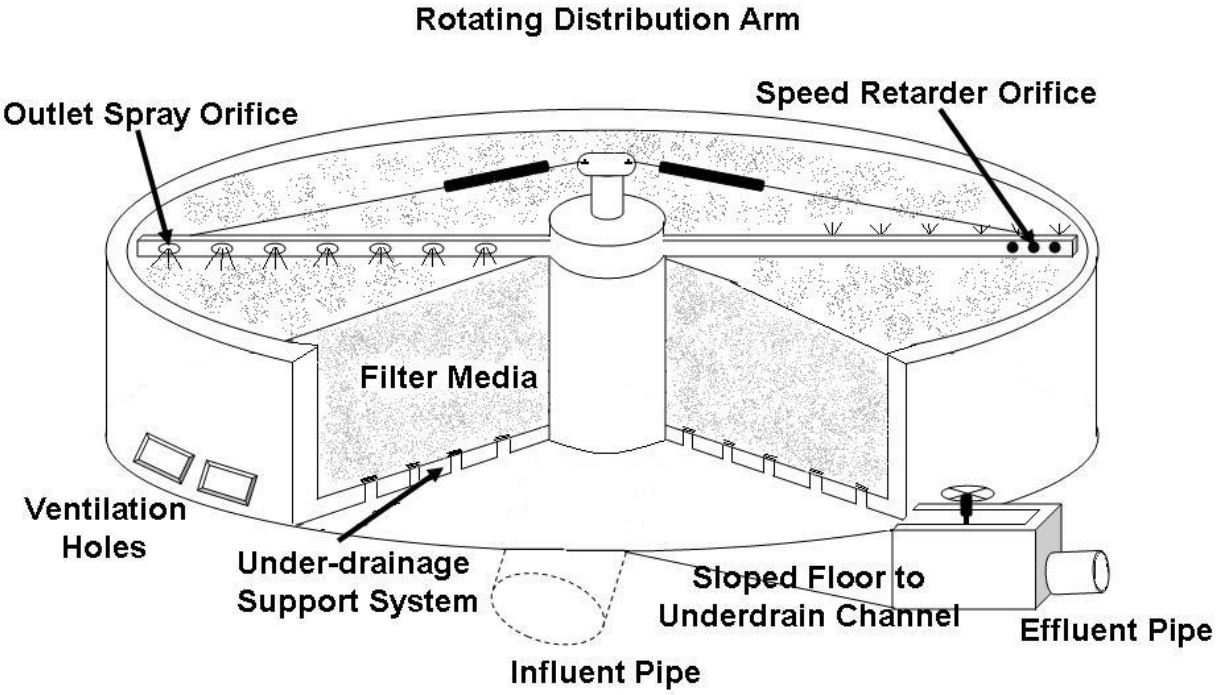
Wastewater temperature also affects receiving waters. Hot water, for example, which is a byproduct of many manufacturing processes, can be a pollutant. When discharged in large quantities, it can raise the temperature of receiving streams locally and disrupt the natural balance of aquatic life.

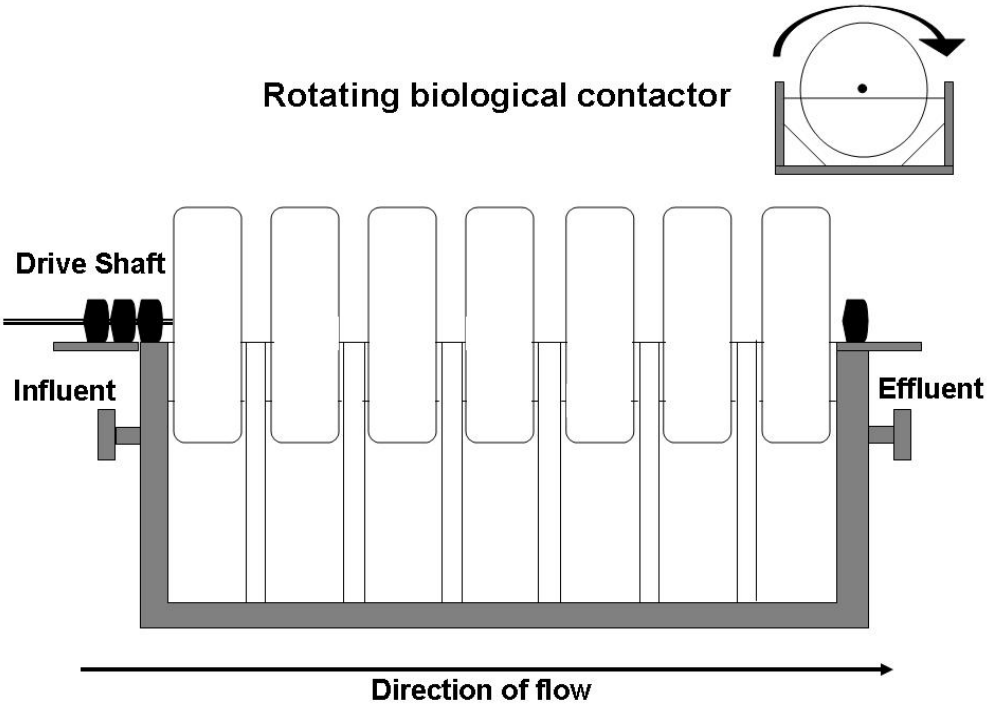
pH
The acidity or alkalinity of wastewater affects both treatment and the environment. Low pH indicates increasing acidity while a high pH indicates increasing alkalinity (a pH of 7 is neutral). The pH of wastewater needs to remain between 6 and 9 to protect organisms. Acids and other substances that alter pH can inactivate treatment processes when they enter wastewater from industrial or commercial sources.

This operator is splitting the sample for bacteriological analysis. Always wear gloves for your and others' safety. We've all seen the operator holds a sandwich in one hand while working in the lab, or the operator does not wear gloves at all. I personally like the operator who can smoke a cigarette or eat while sampling. Hey, why don't you just drink the sample when you are finished? You've already got a free taste of sample. We all get a free-taste-sample working in this industry. Believe or not, I had some terrible experiences, and so might you. I've have been soaked head to toe with mixed liquor, but after a few dozen times, I got used to it. Wastewater samples will make you resistant to any known disease, well, you have to pay the price and get sick for a short period, but after the first 6 months, you can endure almost anything.

My advice is to know how to protect yourself and be careful. Get your hepatitis shots, wear PPE, and don't freak out with a spill or mishap. Always be prepared for the worst. Bring extra clothes to work and soap too.

Free-taste-sample is one of the less known benefits for working at a wastewater facility. Tasty for sure! Guaranteed to curl your toes, you'll never complain again about anything else in life.


Rotating Distribution Arm
Outlet Spray Orifice
Speed Retarder Orifice
Filter Media
Ventilation Holes
Under-drainage Support System
Sloped Floor to Underdrain Channel
Effluent Pipe
Influent Pipe


Rotating biological contactor
Drive Shaft
Influent
Effluent
Direction of flow

Total Dissolved Solids

Water is a good solvent and picks up impurities easily. Pure water is tasteless, colorless, and odorless and is often called the universal solvent. Dissolved solids refer to any minerals, salts, metals, cations or anions dissolved in water. Total dissolved solids (TDS) comprise inorganic salts (principally calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulfates) and some small amounts of organic matter that are dissolved in water.

TDS in drinking-water originate from natural sources, sewage, urban run-off, industrial wastewater, and chemicals used in the water treatment process, and the nature of the piping or hardware used to convey the water, i.e., the plumbing. In the United States, elevated TDS has been due to natural environmental features such as: mineral springs, carbonate deposits, salt deposits, and sea water intrusion, but other sources may include: salts used for road de-icing, anti-skid materials, drinking water treatment chemicals, stormwater and agricultural runoff, and point/non-point wastewater discharges.

In general, the total dissolved solids concentration is the sum of the cations (positively charged) and anions (negatively charged) ions in the water. Therefore, the total dissolved solids test provides a qualitative measure of the amount of dissolved ions, but does not tell us the nature or ion relationships.

In addition, the test does not provide us insight into the specific water quality issues, such as: Elevated Hardness, Salty Taste, or Corrosiveness. Therefore, the total dissolved solids test is used as an indicator test to determine the general quality of the water.

Total Solids

The term "total solids" refers to matter suspended or dissolved in water or wastewater, and is related to both specific conductance and turbidity.

Total solids (also referred to as total residue) are the term used for material left in a container after evaporation and drying of a water sample. Total Solids includes both total suspended solids, the portion of total solids retained by a filter and total dissolved solids, the portion that passes through a filter (American Public Health Association, 1998).

Total solids can be measured by evaporating a water sample in a weighed dish, and then drying the residue in an oven at 103 to 105° C. The increase in weight of the dish represents the total solids. Instead of total solids, laboratories often measure total suspended solids and/or total dissolved solids.

Total Suspended Solids (TSS)
Total Suspended Solids (TSS) are solids in water that can be trapped by a filter. TSS can include a wide variety of material, such as silt, decaying plant and animal matter, industrial wastes, and sewage. High concentrations of suspended solids can cause many problems for stream health and aquatic life.

High TSS can block light from reaching submerged vegetation. As the amount of light passing through the water is reduced, photosynthesis slows down. Reduced rates of photosynthesis causes less dissolved oxygen to be released into the water by plants. If light is completely blocked from bottom dwelling plants, the plants will stop producing oxygen and will die. As the plants are decomposed, bacteria will use up even more oxygen from the water. Low dissolved oxygen can lead to fish kills.

Sampling downstream from a wastewater plant's discharge point.

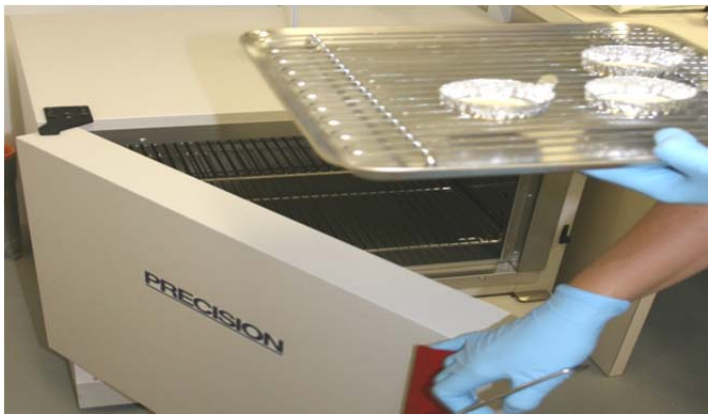
High TSS can also cause an increase in surface water temperature, because the suspended particles absorb heat from sunlight. This can cause dissolved oxygen levels to fall even further (because warmer waters can hold less DO), and can harm aquatic life in many other ways, as discussed in the temperature section. (The decrease in water clarity caused by TSS can affect the ability of fish to see and catch food.

Suspended sediment can also clog fish gills, reduce growth rates, decrease resistance to disease, and prevent egg and larval development. When suspended solids settle to the bottom of a water body, they can smother the eggs of fish and aquatic insects, as well as suffocate newly hatched insect larvae. Settling sediments can fill in spaces between rocks which could have been used by aquatic organisms for homes.

Dead fish in lake using reclaimed water.

High TSS in a water body can often mean higher concentrations of bacteria, nutrients, pesticides, and metals in the water. These pollutants may attach to sediment particles on the land and be carried into water bodies with storm water. In the water, the pollutants may be released from the sediment or travel farther downstream. High TSS can cause problems for industrial use, because the solids may clog or scour pipes and machinery.

Measurement of Total Suspended Solids
To measure TSS, the water sample is filtered through a pre-weighed filter. The residue retained on the filter is dried in an oven at 103 to 105° C until the weight of the filter no longer changes. The increase in weight of the filter represents the total suspended solids. TSS can also be measured by analyzing for total solids and subtracting total dissolved solids.



Total Dissolved Solids (TDS) are solids in water that can pass through a filter (usually with a pore size of 0.45 micrometers). TDS is a measure of the amount of material dissolved in water. This material can include carbonate, bicarbonate, chloride, sulfate, phosphate, nitrate, calcium, magnesium, sodium, organic ions, and other ions. A certain level of these ions in water is necessary for aquatic life. Changes in TDS concentrations can be harmful because the density of the water determines the flow of water into and out of an organism's cells (Mitchell and Stapp, 1992). However, if TDS concentrations are too high or too low, the growth of many aquatic lives can be limited, and death may occur.

Similar to TSS, high concentrations of TDS may also reduce water clarity, contribute to a decrease in photosynthesis, combine with toxic compounds and heavy metals, and lead to an increase in water temperature. TDS is used to estimate the quality of drinking water, because it represents the amount of ions in the water. Water with high TDS often has a bad taste and/or high water hardness, and could result in a laxative effect.

The TDS concentration of a water sample can be estimated from specific conductance if a linear correlation between the two parameters is first established. Depending on the chemistry of the water, TDS (mg/l) can be estimated by multiplying specific conductance (micromhos/cm) by a factor between 0.55 and 0.75. TDS can also be determined by measuring individual ions and adding them up.

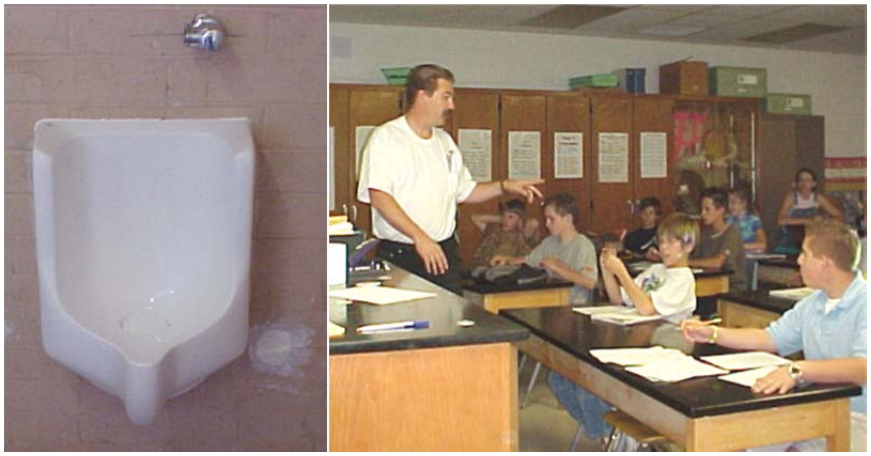
Conductivity Meter

Flow

Whether a system serves a single home or an entire community, it must be able to handle fluctuations in the quantity and quality of wastewater it receives to ensure proper treatment is provided at all times. Systems that are inadequately designed or hydraulically overloaded may fail to provide treatment and allow the release of pollutants to the environment.

To design systems that are both as safe and as cost-effective as possible, engineers must estimate the average and maximum (peak) amount of flows generated by various sources. Because extreme fluctuations in flow can occur during different times of the day and on different days of the week, estimates are based on observations of the minimum and maximum amounts of water used on an hourly, daily, weekly, and seasonal basis. The possibility of instantaneous peak flow events that result from several or all water-using appliances or fixtures being used at once also is taken into account.

The number, type, and efficiency of all water-using fixtures and appliances at the source is factored into the estimate (for example, the number and amount of water normally used by faucets, toilets, and washing machines), as is the number of possible users or units that can affect the amount of water used (for example, the number of residents, bedrooms, customers, students, patients, seats, or meals served).



Waterless urinals are reducing water use but are concentrating the wastestream. Water conservation education is now taught at schools and this too is affecting our flow dynamics and MO's. Anything new always affects the bugs and no one cares but us.

According to studies, water use in many homes is lowest from about midnight to 5 a.m., averaging less than one gallon per person per hour, but then rises sharply in the morning around 6 am to a little over 3 gallons per person per hour. During the day, water use drops off moderately and rises again in the early evening hours. Weekly peak flows may occur in some homes on weekends, especially when all adults work during the week. In U.S. homes, average water use is approximately 45 gallons per person per day, but may range from 35 to 60 gallons or more.

Peak flows at stores and other businesses typically occur during business hours and during meal times at restaurants. Rental properties, resorts, and commercial establishments in tourist areas may have extreme flow variations seasonally. Estimating flow volumes for centralized treatment systems is a complicated task, especially when designing a new treatment plant in a community where one has never existed previously.

Engineers must allow for additional flows during wet weather due to inflow and infiltration of extra water into sewers. Excess water can enter sewers through leaky manhole covers and cracked pipes and pipe joints, diluting wastewater, which affects its overall characteristics. This can increase flows to treatment plants sometimes by as much as three or four times the original design load.

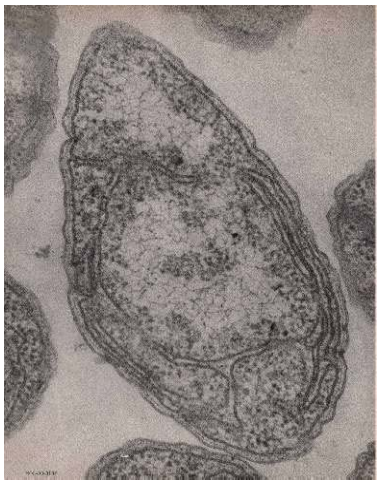
Grout is used to prevent infiltration into manholes.

The main focus of wastewater treatment plants is to reduce the BOD and COD in the effluent discharged to natural waters, meeting state and federal discharge criteria. Wastewater treatment plants are designed to function as "microbiology farms," where bacteria and other microorganisms are fed oxygen and organic waste. Treatment of wastewater usually involves biological processes such as the activated sludge system in the secondary stage after preliminary screening to remove coarse particles and primary sedimentation that settles out suspended solids. These secondary treatment steps are generally considered environmental biotechnologies that harness natural self-purification processes contained in bioreactors for the biodegradation of organic matter and bioconversion of soluble nutrients in the wastewater.

Application Specific Microbiology

Each wastewater stream is unique, and so too are the community of microorganisms that process it. This "application-specific microbiology" is the preferred methodology in wastewater treatment affecting the efficiency of biological nutrient removal. The right laboratory prepared bugs are more efficient in organics removal if they have the right growth environment. This efficiency is multiplied if microorganisms are allowed to grow as a layer of biofilm on specifically designed support media. In this way, optimized biological processing of a waste stream can occur. To reduce the start-up phase for growing a mature biofilm one can also purchase "application specific bacterial cultures" from appropriate microbiology vendors.

Draining Biofilm



Nitrosomonas europaea

Advanced Methods of Wastewater Treatment
As our country and the demand for clean water have grown, it has become more important to produce cleaner wastewater effluents, yet some contaminants are more difficult to remove than others. The demand for cleaner discharges has been met through better and more complete methods of removing pollutants at wastewater treatment plants, in addition to pretreatment and pollution prevention which helps limit types of wastes discharged to the sanitary sewer system.

Currently, nearly all WWTPs provide a minimum of secondary treatment. In some receiving waters, the discharge of secondary treatment effluent would still degrade water quality and inhibit aquatic life. Further treatment is needed.

Discharge point from a wastewater plant into a wetlands project.

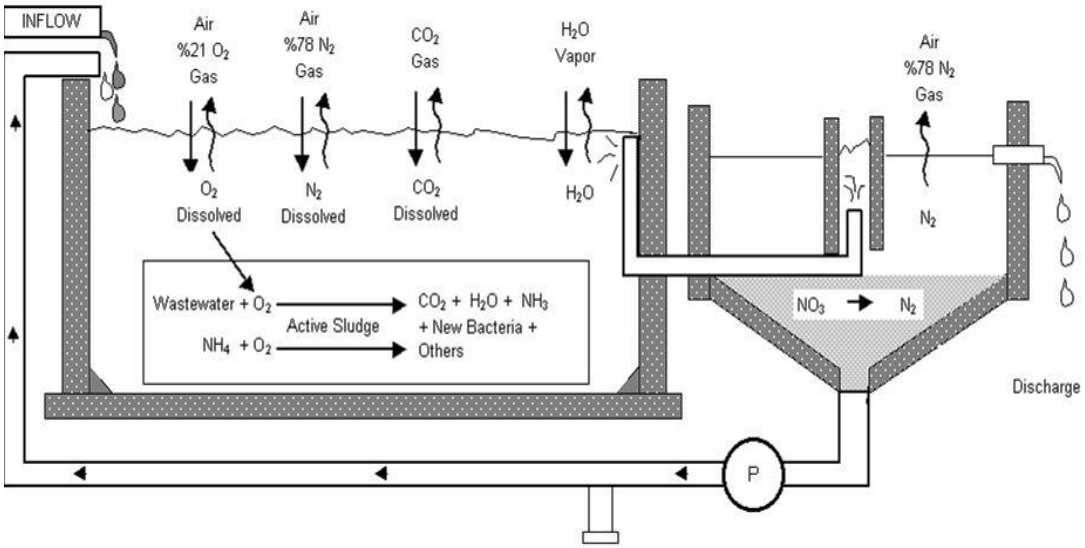
Advanced Treatment Technologies
Treatment levels beyond secondary are called advanced treatment. Advanced treatment technologies can be extensions of conventional secondary biological treatment to further stabilize oxygen-demanding substances in the wastewater, or to remove nitrogen and phosphorus. Advanced treatment may also involve physical-chemical separation techniques such as adsorption, flocculation/precipitation, membranes for advanced filtration, ion exchange, and reverse osmosis. In various combinations, these processes can achieve any degree of pollution control desired. As wastewater is purified to higher and higher degrees by such advanced treatment processes, the treated effluents can be reused for urban, landscape, and agricultural irrigation, industrial cooling and processing, recreational uses and water recharge, and even indirect augmentation of drinking water supplies.

Nitrogen Control

Nitrogen in one form or another is present in municipal wastewater and is usually not removed by secondary treatment. If discharged into lakes and streams or estuary waters, nitrogen in the form of ammonia can exert a direct demand on oxygen or stimulate the excessive growth of algae. Ammonia in wastewater effluent can be toxic to aquatic life in certain instances. By providing additional biological treatment beyond the secondary stage, nitrifying bacteria present in wastewater treatment can biologically convert ammonia to the non-toxic nitrate through a process known as nitrification. The nitrification process is normally sufficient to remove the toxicity associated with ammonia in the effluent. Since nitrate is also a nutrient, excess amounts can contribute to the uncontrolled growth of algae. In situations where nitrogen must be completely removed from effluent, additional biological process can be added to the system to convert the nitrate to nitrogen gas. We will cover this in much more detail in a few more pages.

Conversion of Nitrate to Nitrogen Gas

The conversion of nitrate to nitrogen gas is accomplished by bacteria in a process known as denitrification. Effluent with nitrogen in the form of nitrate is placed into a tank devoid of oxygen, where carbon-containing chemicals, such as methanol, are added or a small stream of raw wastewater is mixed in with the nitrified effluent. In this oxygen free environment, bacteria use the oxygen attached to the nitrogen in the nitrate form, releasing nitrogen gas. Because nitrogen comprises almost 80 percent of the air in the earth's atmosphere, the release of nitrogen into the atmosphere does not cause any environmental harm.



Biological Phosphorus Control

Like nitrogen, phosphorus is also a necessary nutrient for the growth of algae. Phosphorus reduction is often needed to prevent excessive algal growth before discharging effluent into lakes, reservoirs and estuaries. Phosphorus removal can be achieved through chemical addition and a coagulation-sedimentation process discussed in the following section. Some biological treatment processes called biological nutrient removal (BNR) can also achieve nutrient reduction, removing both nitrogen and phosphorus.

Most of the BNR processes involve modifications of suspended growth treatment systems so that the bacteria in these systems also convert nitrate nitrogen to inert nitrogen gas and trap phosphorus in the solids that are removed from the effluent.

Coagulation-Sedimentation Process

A process known as chemical coagulation-sedimentation is used to increase the removal of solids from effluent after primary and secondary treatment. Solids heavier than water settle out of wastewater by gravity. With the addition of specific chemicals, solids can become heavier than water and will settle.

Alum, lime, or iron salts are chemicals added to the wastewater to remove phosphorus. With these chemicals, the smaller particles 'floc' or clump together into large masses. The larger masses of particles will settle faster when the effluent reaches the next step the sedimentation tank. This process can reduce the concentration of phosphate by more than 95 percent.

Although used for years in the treatment of industrial wastes and in water treatment, coagulation-sedimentation is considered an advanced process because it is not routinely applied to the treatment of municipal wastewater. In some cases, the process is used as a necessary pretreatment step for other advanced techniques. This process produces a chemical sludge, and the cost of disposing of this material can be significant.

Carbon Adsorption

Carbon adsorption technology can remove organic materials from wastewater that resist removal by biological treatment. These resistant, trace organic substances can contribute to taste and odor problems in water, taint fish flesh, and cause foaming and fish kills. Carbon adsorption consists of passing the wastewater effluent through a bed or canister of activated carbon granules or powder which remove more than 98 percent of the trace organic substances. The substances adhere to the carbon surface and are removed from the water. To help reduce the cost of the procedure, the carbon granules can be cleaned by heating and used again.

Granular Carbon

The Use or Disposal of Wastewater Residuals and Biosolids

When pollutants are removed from water, there is always something left over. It may be rags and sticks caught on the screens at the beginning of primary treatment. It may be the solids that settle to the bottom of sedimentation tanks. Whatever it is, there are always residuals that must be reused, burned, buried, or disposed of in some manner that does not harm the environment.

The utilization and disposal of the residual process solids is addressed by the CWA, Resource Conservation and Recovery Act (RCRA), and other federal laws. These Federal laws re-enforce the need to employ environmentally sound residuals management techniques and to beneficially use biosolids whenever possible. We will cover this in much more detail in a few more pages.

Processed Wastewater Solids

Biosolids are processed wastewater solids ("sewage sludge") that meet rigorous standards allowing safe reuse for beneficial purposes. Currently, more than half of the biosolids produced by municipal wastewater treatment systems are applied to land as a soil conditioner or fertilizer and the remaining solids are incinerated or landfilled.

Large solids treatment facility

Ocean Dumping
Ocean dumping of these solids is no longer allowed.

Biosolids Stabilization
Prior to utilization or disposal, biosolids are stabilized to control odors and reduce the number of disease-causing organisms. Sewage solids, or sludge, when separated from the wastewater, still contain around 98 percent water. They are usually thickened and may be dewatered to reduce the volume to be transported for final processing, disposal, or beneficial use.

Dewatering Processes
Dewatering processes include drying beds, belt filter presses, plate and frame presses, and centrifuges. To improve dewatering effectiveness, the solids can be pretreated with chemicals such as lime, ferric chloride, or polymers to produce larger particles which are easier to remove.

Centrifuge

Filter Press

Digestion
Digestion is a form of stabilization where the volatile material in the wastewater solids can decompose naturally and the potential for odor production is reduced. Digestion without air in an enclosed tank (anaerobic solids digestion) has the added benefit of producing methane gas which can be recovered and used as a source of energy. Stabilization of solids may also be accomplished by composting, heat treatments, drying or the addition of lime or other alkaline materials. After stabilization, the biosolids can be safely spread on land.

Land Application
In many areas, biosolids are marketed to farmers as fertilizer. Federal regulation (40 CFR Part 503) defines minimum requirements for such land application practices, including contaminant limits, field management practices, treatment requirements, monitoring, recordkeeping, and reporting requirements. Properly treated and applied biosolids are a good source of organic matter for improving soil structure and help supply nitrogen, phosphorus, and micronutrients that are required by plants.

Biosolids have also been used successfully for many years as a soil conditioner and fertilizer, and for restoring and re-vegetating areas with poor soils due to construction activities, strip mining or other practices. Under this biosolids management approach, treated solids in semi liquid or dewatered form are transported to the soil treatment areas. The slurry or dewatered biosolids, containing nutrients and stabilized organic matter, is spread over the land to give nature a hand in returning grass, trees, and flowers to barren land.

Restoration of the countryside also helps control the flow of acid drainage from mines that endangers fish and other aquatic life and contaminates the water with acid, salts, and excessive quantities of metals.

Incineration
Incineration consists of burning the dried solids to reduce the organic residuals to an ash that can be disposed of or reused. Incinerators often include heat recovery features. Undigested sludge solids have significant fuel value as a result of their high organic content. However, the water content must be greatly reduced by dewatering or drying to take advantage of the fuel potential of the biosolids. For this reason, pressure filtration dewatering equipment is used to obtain biosolids which are sufficiently dry to burn without continual reliance on auxiliary fuels. In some cities, biosolids are mixed with refuse or refuse derived fuel prior to burning. Generally, waste heat is recovered to provide the greatest amount of energy efficiency.

Beneficial Use Products from Biosolids
Heat dried biosolids pellets have been produced and used extensively as a fertilizer product for lawn care, turf production, citrus groves, and vegetable production for many years. Composting of biosolids is also a well-established approach to solids management that has been adopted by a number of communities. The composted peat-like product has shown particular promise for use in the production of soil additives for re-vegetation of topsoil depleted areas, and as a potting soil amendment.

Effective pretreatment of industrial wastes prevents excessive levels of unwanted constituents, such as heavy metals (i.e. cadmium, mercury, and lead) and persistent organic compounds from contaminating the residuals of wastewater treatment and limiting the potential for beneficial use.

Effective stabilization of wastewater residuals and their conversion to biosolid products can be costly. Some cities have produced fertilizers from biosolids which are sold to help pay part of the cost of treating wastewater. Some municipalities use composted, heat dried, or lime stabilized biosolid products on parks and other public areas.

Decentralized (Onsite and Cluster) Systems
A decentralized wastewater system treats sewage from homes and businesses that are not connected to a centralized wastewater treatment plant. Decentralized treatment systems include onsite systems and cluster systems. An onsite system is a wastewater system relying on natural processes, although sometimes containing mechanical components, to collect, treat, disperse or reclaim wastewater from a single dwelling or building. A septic tank and soil adsorption field is an example of an onsite system.

A wastewater collection and treatment system under some form of common ownership that collects wastewater from two or more dwellings or buildings and conveys it to a treatment and dispersal system located on a suitable site near the dwellings or buildings is a cluster system.

Decentralized systems include those using alternative treatment technologies like media filters, constructed wetland systems, aerobic treatment units, and a variety of soil dispersal systems. Soil dispersal systems include pressure systems such as low pressure pipe and drip dispersal systems. These systems treat and disperse relatively small volumes of wastewater, and are generally are found in rural and suburban areas.

While septic tanks and soil absorption systems have significant limitations, decentralized systems can effectively protect water quality and public health from groundwater and surface water contamination if managed properly (i.e. properly sited, sized, designed, installed, operated, and maintained). Nitrate concentrations in groundwater that exceed the drinking water standards can cause health problems.

Onsite Treatment
Onsite wastewater systems contain three components: a treatment unit which treats water prior to dispersal into the environment; a soil dispersal component which assures that treated water is released into the environment at a rate which can be assimilated; and a management system which assures proper long term operation of the complete system.

Disinfection of the treated effluent may be provided prior to dispersal. A typical onsite system consists of a septic tank followed by an effluent distribution system. Alternative treatment systems include aerobic treatment and sand filtration systems. We will cover this in much more detail in a few more pages.

Conventional Septic Tanks
A septic tank is a tank buried in the ground used to treat sewage without the presence of oxygen (anaerobic). The sewage flows from the plumbing in a home or small business establishment into the first of two chambers, where solids settle out. The liquid then flows into the second chamber. Anaerobic bacteria in the sewage break down the organic matter, allowing cleaner water to flow out of the second chamber. The liquid typically discharges through a subsurface distribution system. Periodically, the solid matter in the bottom of the tank, referred to as septage, must be removed and disposed of properly.

Aerobic Treatment Units
Aerobic treatment units are also used to provide onsite wastewater treatment. They are similar to septic tanks, except that air is introduced and mixed with the wastewater inside the tank. Aerobic (requiring oxygen) bacteria consume the organic matter in the sewage. As with the typical septic system, the effluent discharge from an aerobic system is typically released through a sub-surface distribution system or may be disinfected and discharged directly to surface water. Aerobic treatment units also require the removal and proper disposal of solids that accumulate in the tank.

Media Filters
Media filters are used to provide further treatment of septic tank effluent, and provide high levels of nitrification. They can be designed to pass the effluent once or multiple times through the media bed. Media, such as sand, acts as a filter. The media is placed two to three feet deep above a liner of impermeable material such as plastic or concrete. Septic tank effluent is applied to the filter surface in intermittent doses and is further treated as it slowly trickles through the media. In most media filters, wastewater is collected in an underdrain then either pumped back to the filter bed or to other types of treatment. We will cover this in much more detail in a few more pages.

Dispersal Approaches
Traditional onsite systems include treatment units followed by a drainfield or absorption field. Wastewater from the treatment unit is dispersed through a suitable soil layer where it receives additional treatment by the soil microorganisms and filtering properties of the soil. If the soil is unsuitable for the installation of a soil absorption field, alternative methods can be used to further treat or distribute the treated effluent. The most common alternative dispersal systems include low-pressure pipe, mounds, drip disposal, and evapotranspiration beds.

Absorption Field
When soil conditions permit, the most common method to disperse septic tank or aerobic system effluent is an absorption field consisting of a series of perforated parallel pipes laid in trenches on gravel or crushed stone or as a direct discharge to the soil through trenches.

Typically, effluent flows into the absorption field from a distribution box which maintains an even flow of effluent to the absorption field. From there, the effluent drains through the stone and into the soil which provides further treatment.

Mound System
When the soil is not conducive to percolation or when the groundwater level is high, a mound system is commonly used. A mound system is a distribution system constructed above the original ground level by using granular material such as sand and gravel to receive the septic tank effluent before it flows to the native soil below. The effluent flows to a dosing tank that is equipped with a pump. Here the effluent is stored until there is sufficient liquid. Once the liquid is pumped out, it moves evenly throughout the mound before reaching less permeable soil or groundwater. The granular material acts as a treatment medium and improves the removal of pollutants in ways that may not be provided by substandard native soils.

Drip Dispersal System
Where soils are very thin or have reduced permeability, drip dispersal systems can be utilized. The typical drip system operates like drip irrigation at a moderately high pressure. The components of a drip system include filters to remove solids, a network of drip tubes to disperse liquid into soil, tanks to hold liquid, and controllers to regulate the flow to the drip system.

Evapotranspiration Beds
Evapotranspiration (ET) bed is an onsite dispersal system where pretreated wastewater evaporates from the soil surface or is transpired by plants into the atmosphere. Usually, ET beds are used in arid climates and there is no discharge either to surface or groundwater. Vegetation is planted on the surface of the sand bed to improve the transpiration process and landscaping enhances the aesthetics of the bed.

Management of Decentralized Systems

Ensuring performance of decentralized wastewater treatment systems is an issue of national concern because these systems are a permanent component of our nation's wastewater infrastructure. Twenty five percent of households nationwide and one-third of the new homes being constructed are served by onsite systems. Many of the existing systems do not perform adequately due to a lack of management. Therefore, the EPA promotes the sustained management of decentralized wastewater systems to enhance their performance and reliability. The EPA strongly encourages communities to establish management programs for the maintenance of onsite systems in addition to improving local requirements for onsite system siting and system design.

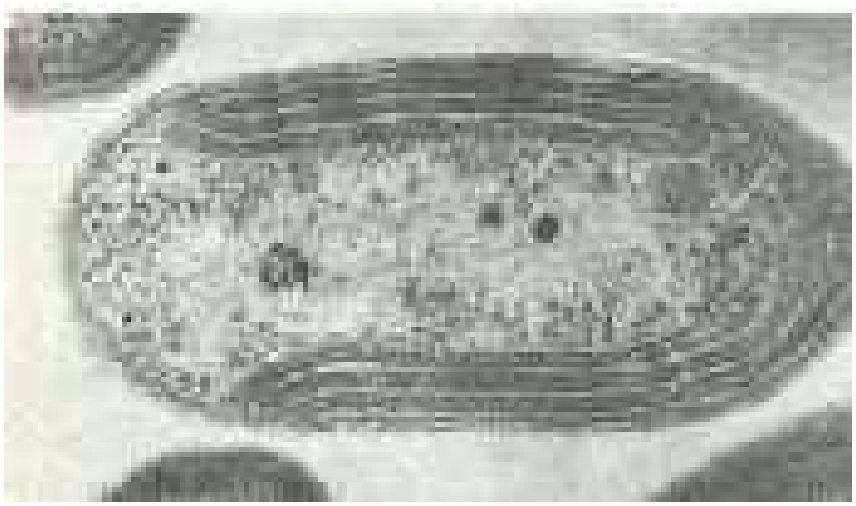
Communities benefit from effective onsite system management programs by enjoying improved protection of public health and local surface water and groundwater resources, preserving rural areas, protecting property owners' investments through increased system service life, and avoiding the need to finance costly central wastewater collection and treatment systems.

Dispose of Household Hazardous Wastes Safely
Many household products are potentially hazardous to people and the environment and never should be flushed down drains, toilets, or storm sewers. Treatment plant workers can be injured and wastewater systems can be damaged as a result of improper disposal of hazardous materials. Other hazardous chemicals cannot be treated effectively by municipal wastewater systems and may reach local drinking water sources. When flushed into septic systems and other onsite systems, they can temporarily disrupt the biological processes in the tank and soil absorption field, allowing hazardous chemicals and untreated wastewater to reach groundwater.

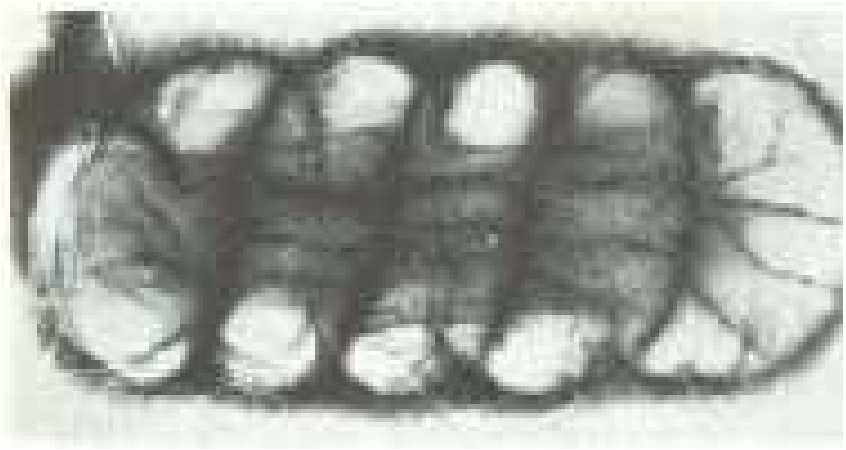
Some examples of hazardous household materials include motor oil, transmission fluid, antifreeze, paint, paint thinner, varnish, polish, wax, solvents, pesticides, rat poison, oven cleaner, and battery fluid. Many of these materials can be recycled or safely disposed of at community recycling centers.

A drive-thru household hazardous waste collection site, trying to keep the toxic material out of the sewer system. These workers usually get to keep lots of goodies and take the materials home while at the same time keeping the bad stuff from upsetting the plant. I worked a day at one of these facilities and I was amazed with the chemicals that people keep around the home, example, 1 pound of liquid Mercury and another had a bottle of Sodium Cyanide.

Top photograph, a clarifier's raking mechanism.
Bottom, scum armature equipment.



Nitrobacter winogradskyi



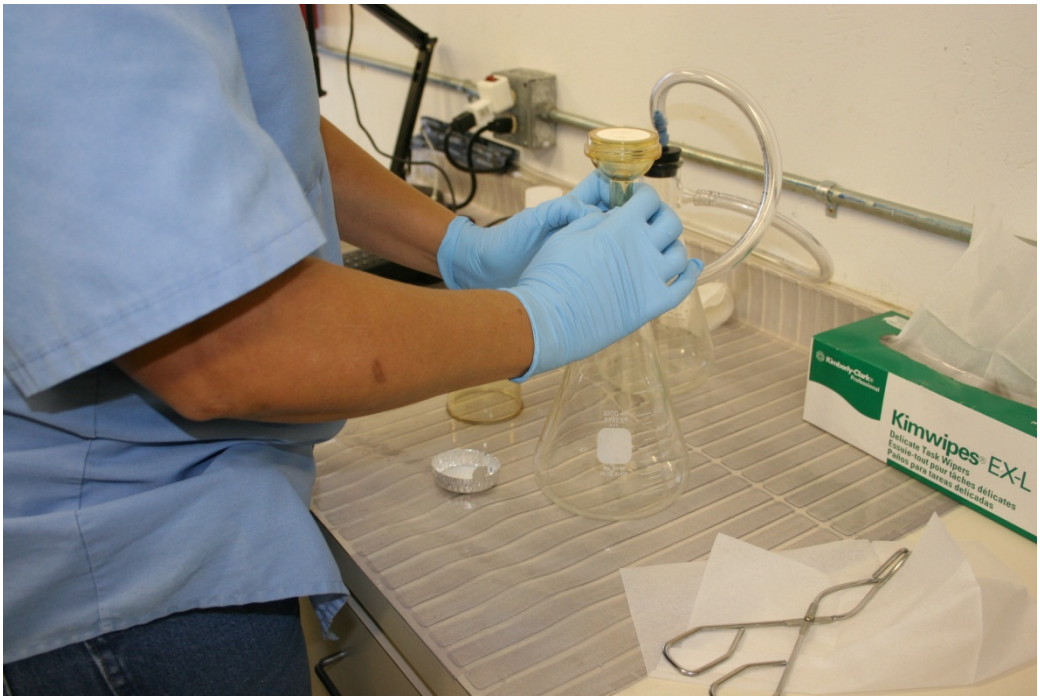
Nitrospira gracilis

Of all biological waste treatment methods, aerobic digestion is the most widespread process used throughout the world (more than 95%).

Nature gives, takes and does everything in-between. Nowhere is this better exemplified than the biological solution it offers to mankind's waste problems. An illustration of nature's work is its influence on the constant cycle of biological waste treatment. Microorganisms, like all living things, require food for growth.

Biological sewage treatment consists of many different microorganisms, mostly bacteria, carrying out a stepwise, continuous, sequential attack on the organic compounds found in wastewater and upon which the microbes feed.

Aerobic digestion of waste is the natural biological degradation and purification process in which bacteria that thrive in oxygen-rich environments break down and digest the waste. During this oxidation process, pollutants are broken down into carbon dioxide (CO_2), water (H_2O), nitrates, sulfates and biomass (micro-organisms). By optimizing the oxygen supply with so called aerators the process can be significantly accelerated.



Total dissolved solids - The weight per unit volume of all volatile and non-volatile solids dissolved in a water or wastewater after a sample has been filtered to remove colloidal and suspended solids.

Water Quality Criteria

Many types of microscopic plants and animals, such as plankton, water beetles, and insects that live in or on the water, serve as food for small fish. Small fish are eaten by larger fish which, in turn, are consumed by even larger fish. These large fish may ultimately be consumed by humans. All life along the food chain is dependent on the water environment and it is for this reason that the quality of the nation's surface waters must be protected.

The Clean Water Act directs the EPA to develop criteria for water quality that accurately reflect the latest scientific knowledge about the effects of pollutants on aquatic life and human health. In developing these criteria, the EPA examines the effects of specific pollutants on plankton, fish, shellfish, wildlife, plant life, aesthetics, and recreation in any body of water. This includes specific information on the concentration and dispersal of pollutants through biological, physical, and chemical processes as well as the effects of pollutants on biological communities as a whole.

States may use the criteria that are developed by the EPA to help set water quality standards that protect the uses of their waters or they may develop their own water quality criteria. The EPA publishes human health and aquatic life criteria and is currently developing sediment and biological criteria. These criteria are complementary; each is designed to protect specific types of living organisms or ecological systems from the adverse effects of pollution.

Human Health Criteria
People can potentially be exposed to water pollutants when they drink untreated surface water or eat fish, shellfish, or wildlife that have been contaminated by pollutants in surface waters. To reduce the risk to humans from these sources, the EPA scientists research information to determine the levels at which specific chemicals are not likely to adversely affect human health. The EPA publishes these levels as human health criteria that the states use, along with other information, to set allowable concentrations of pollutants in their water quality standards. In this way, the EPA and the states work together to protect people from exposure to harmful pollutants in surface waters.

For an in-depth look visit:
http://epa.gov/waterscience/criteria/humanhealth/

Aquatic Life Criteria
Aquatic life criteria provide protection for plants and animals that are found in surface waters. The EPA develops these criteria as numeric limits on the amounts of chemicals that can be present in river, lake, or stream water without harm to aquatic life. Aquatic life criteria are designed to provide protection for both freshwater and saltwater aquatic organisms from the effects of acute (short term) and chronic (long term) exposure to potentially harmful chemicals. Aquatic life criteria are based on toxicity information and are developed to protect aquatic organisms from death, slower growth, reduced reproduction, and the accumulation of harmful levels of toxic chemicals in their tissues that may adversely affect consumers of such organisms.

For an in-depth look visit:
http://epa.gov/waterscience/criteria/aqlife.html

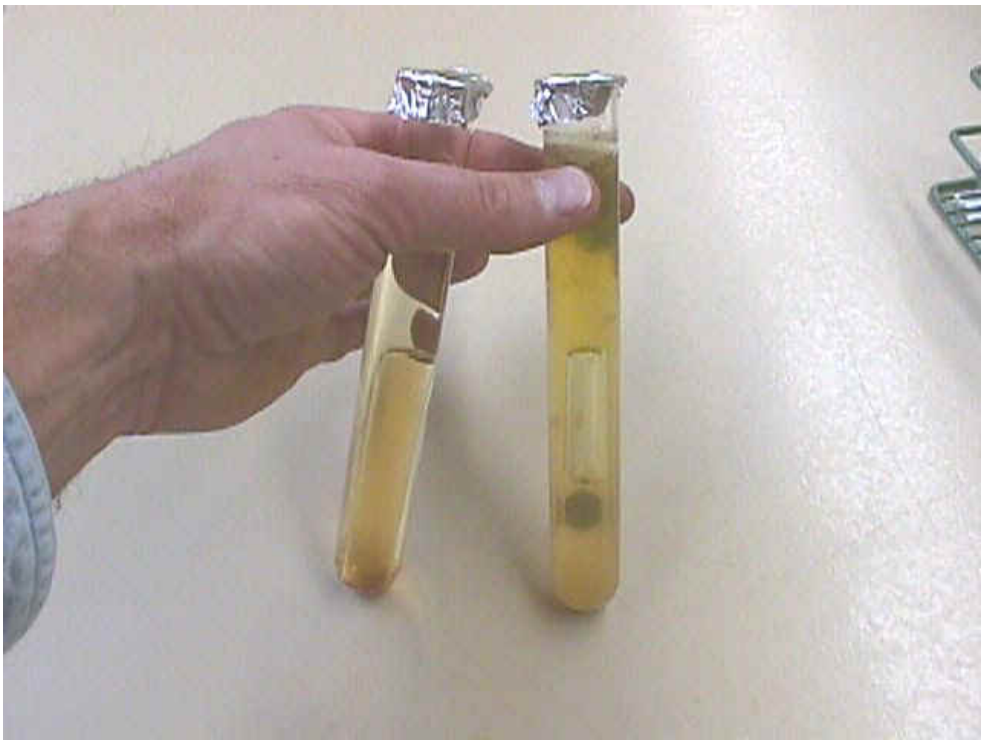
Sediment Quality Criteria Guidance
In a healthy aquatic community, sediments provide a habitat for many living organisms. Worms, plants, and tiny microorganisms living in or on the sediment sustain the fish and shellfish that, in turn, nourish larger fish, wildlife, and man.

Pollutants in the Sediment
Controlling the concentration of pollutants in the sediment helps to protect bottom dwelling species and prevents harmful toxins from moving up the food chain and accumulating in the tissue of animals at progressively higher levels. This is particularly important at the lower levels of the food chain because the concentration of many pollutants may increase at each link in the food chain. A pollutant level in the sediment that does not harm snails of small fish may bioaccumulate in the food chain and become very harmful to larger fish, birds, mammals, wildlife, and people.

The EPA develops sediment quality criteria guidance on the concentrations or amounts of individual chemicals that can be present in river, lake, or stream sediments and still protect sediment-dwelling organisms and ultimately animals higher in the food chain from the harmful effects of toxic pollutants.

For an in-depth look visit:
http://epa.gov/waterscience/criteria/sediment/

Biological Criteria
A water body in its natural condition is free from the harmful effects of pollution, habitat loss, and other negative stressors. It is characterized by a particular biological diversity and abundance of organisms. This biological integrity--or natural structure and function of aquatic life--can be dramatically different in various types of water bodies in different parts of the country. Because of this, the EPA is developing methodologies that states can use to assess the biological integrity of their waters and, in so doing, set protective water quality standards. These methodologies will describe scientific methods for determining a particular aquatic community's health and for maintaining optimal conditions in various bodies of water.



Standard Total Coliform Fermentation Technique

Summary
The goal of all biological wastewater treatment systems is to remove the non-settling solids and the dissolved organic load from the effluents by using microbial populations. Biological treatments are generally part of secondary treatment systems. The microorganisms used are responsible for the degradation of the organic matter and the stabilization of organic wastes. With regard to the way in which they utilize oxygen, they can be classified into aerobic (require oxygen for their metabolism), anaerobic (grow in absence of oxygen) and facultative (can proliferate either in absence or presence of oxygen although using different metabolic processes). Most of the micro-organisms present in wastewater treatment systems use the organic content of the wastewater as an energy source to grow, and are thus classified as heterotrophes from a nutritional point of view. The population active in a biological wastewater treatment is mixed, complex and interrelated.

Genera
By example, in a single aerobic system, members of the genera *Pseudomonas, Nocardia, Flavobacterium, Achromobacter and Zooglea* may be present, together with filamentous organisms *(Beggioata* and *Spaerotilus* among others). In a well-functioning system, protozoas and rotifers are usually present and are useful in consuming dispersed bacteria or non-settling particles. More extensive description and treatment of the microbiology of wastewater treatment systems are given elsewhere (Stanier, 1976).

The organic load present is incorporated in part as biomass by the microbial populations, and almost all the rest is liberated as gas (carbon dioxide (CO_2) if the treatment is aerobic, or carbon dioxide plus methane (CH_4) if the process is anaerobic) and water. In fisheries wastewaters the non- biodegradable portion is very low.

Unless the cell mass formed during the biological treatment is removed from the wastewater (e.g., by sedimentation or other treatment described in the previous section), the treatment is largely incomplete, because the biomass itself will appear as organic load in the effluent and the only pollution reduction accomplished is that fraction liberated as gases.

The biological treatment processes used for wastewater treatment are broadly classified as aerobic in which aerobic and facultative micro-organisms predominate or anaerobic which use anaerobic micro-organism.

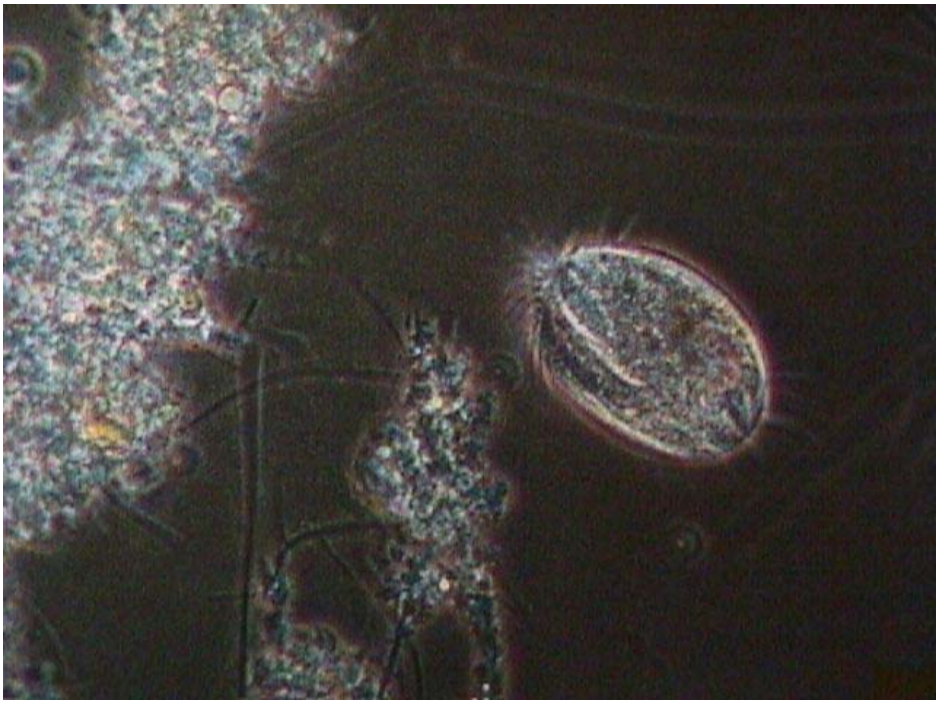
If the microorganisms or Bugs are suspended in the wastewater during biological operation, the operations are "called suspended growth processes", while the micro-organisms that are attached to a surface over which they grow are called "attached growth processes".

This section explains the principles and main characteristics of the most common processes in each case.

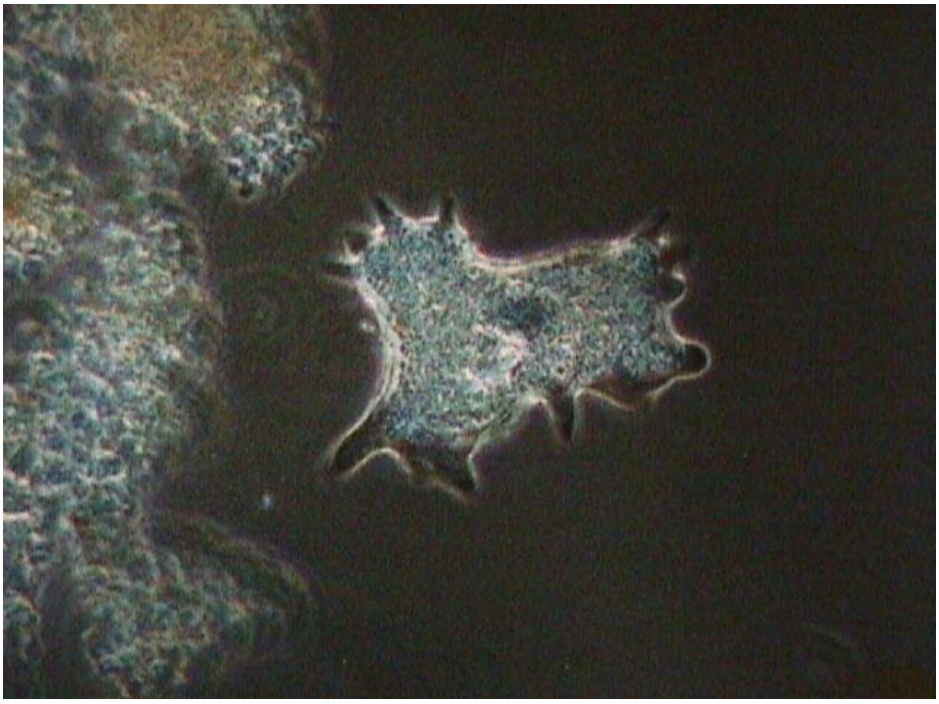
Aerobic Processes
In these, the reactions occurring can be summarized as:

organic load + oxygen + more cells + CO_2 + H_2O

In fisheries wastewaters, the need for addition of nutrients (the most common being nitrogen and phosphorus) seldom appears, but an adequate provision of oxygen is essential for successful operation of the systems. The most common aerobic processes are: activated sludge systems, lagoons, trickling filters and rotating disk contactors. These aerobic processes are described, together with the devices used for aeration.



Ciliate above, Amoeba, below.



The Microlife or the Microorganisms

We talked about the basic components and designs of wastewater treatment now let's look at the main "Team Players". Your process will respond to whatever direction you give it. You can run your plant (the team) to always try for the better or be content with the way it is. To get the best, it takes work!

Most activated sludge processes are used to degrade carbonaceous BOD. It is also possible to design and/or operate the basic system to oxidize ammonia (nitrification).

Many plants are now designed to achieve nitrification. Other system modifications include phosphorus removal and biological denitrification. Activated sludge plants are usually designed from pilot plant and laboratory studies.

From this approach, it is possible to design a process based on the amount of time the sludge spends in the system, generally termed mean cell residence time (MCRT), or on the amount of food provided to the bacteria in the aeration tank (the food-to-microorganism ratio, F/M). What does this mean?

Suppose a person ate 10 pounds of hot dogs (BOD) and weighed 200 pounds (MLSS).

What is the ratio of food to weight?

It would be 10 lbs. to 200 lbs. If we divide 200 into 10, the ratio is .05 or 5%.

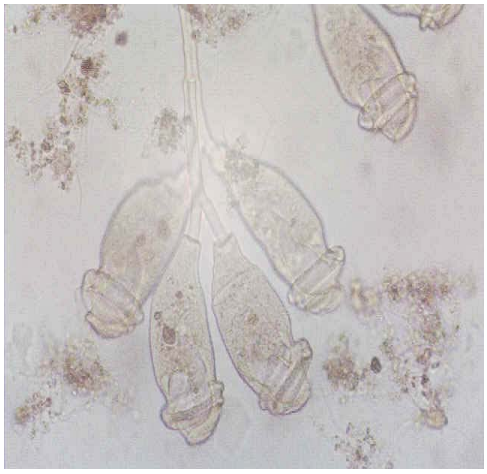
Activated Sludge Aeration Basin, you can tell by the bubbles.

F/M and MCRT

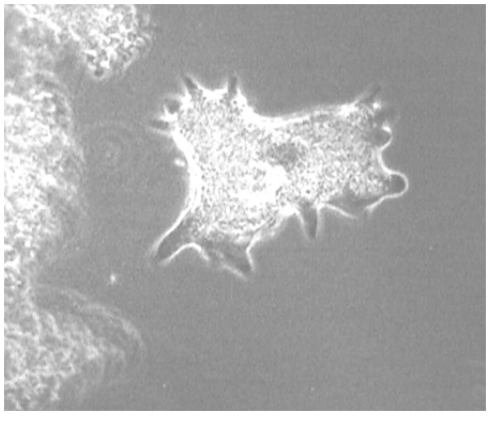
The following are some general statements about F/M and MCRT assuming that the environmental conditions are properly controlled.

a. The optimum operating point of either helps obtain the desired effluent concentration.
b. Both provide a means for maintaining the best effluent and sludge quality.
c. Both techniques attempt to regulate rate of growth, metabolism, and stabilization of food matter.
d. Both techniques indicate the solids level needed to stabilize the food and attain sludge quality.
e. The desired solids level is controlled by wasting.
    1. To maintain – waste amount of net daily
    2. To increase – decrease waste rate
    3. To decrease – increase waste rate
f. They are interrelated so changing one control changes the other.
g. Once the control point is set, it should remain constant until change in effluent or sludge quality requires a change.

The operating control point is that point when the best effluent and sludge quality is obtained for the existing conditions.



Ciliate



Amoeba

Microorganisms in Lagoons

Before we look at the bugs themselves, let's look at eating habits. Have you ever met a person who was a picky eater?

You have people who will put their noses up at some things and others who would eat anything. Predators typically eat from a narrow set of prey, while omnivores and scavengers eat from a broader food selection.

- Swimming and gliding ciliates engulf bacteria or other prey.
- Stalked ciliates attach to the biomass and vortex suspended bacteria into their gullets, while crawlers break bacteria loose from the floc surface.
- Predators feed mostly on stalked and swimming ciliates. The omnivores, such as most rotifers, eat whatever is readily available, while the worms feed on the floc or prey on larger organisms. Microorganisms are directly affected by their treatment environment.
- Changes in food, dissolved oxygen, temperature, pH, total dissolved solids, sludge age, presence of toxins, and other factors create a dynamic environment for the treatment organisms.

Food (organic loading) regulates microorganism numbers, diversity, and species when other factors are not limiting. The relative abundance and occurrence of organisms at different loadings can reveal why some organisms are present in large numbers while others are absent.

Aerobic Bacteria
The aerobic bacteria that occur are similar to those found in other treatment processes such as activated sludge. Three functional groups occur: freely dispersed, single bacteria; floc-forming bacteria; and filamentous bacteria. All function similarly to oxidize organic carbon (BOD) to produce CO_2 and new bacteria (new sludge).

Many bacterial species that degrade wastes grow as single bacteria dispersed in the wastewater. Although these readily oxidize BOD, they do not settle and hence often leave the system in the effluent as solids (TSS). These tend to grow in lagoons at high organic loading and low oxygen conditions. More important are the floc-forming bacteria, those that grow in a large aggregate (floc) due to exocellular polymer production (the glycocalyx).

This growth form is important as these flocs degrade BOD and settle at the end of the process, producing a low TSS effluent.

A number of filamentous bacteria occur in lagoons, usually at specific growth environments. These generally do not cause any operational problems in lagoons, in contrast to activated sludge where filamentous bulking and poor sludge settling is a common problem.

Most heterotrophic bacteria have a wide range in environmental tolerance and can function effectively in BOD removal over a wide range in pH and temperature. Aerobic BOD removal generally proceeds well from pH 6.5 to 9.0 and at temperatures from 3-4°C to 60-70°C (mesophilic bacteria are replaced by thermophilic bacteria at temperatures above 35°C). BOD removal generally declines rapidly below 3-4°C and ceases at 1-2°C.A very specialized group of bacteria occurs to some extent in lagoons (and other wastewater treatment systems) that can oxidize ammonia via nitrite to nitrate, termed nitrifying bacteria. These bacteria are strict aerobes and require a redox potential of at least +200 m V (Holt et al., 1994).

Aerated lagoons

The aerated lagoons are basins, normally excavated in earth and operated without solids recycling into the system. This is the major difference with respect to activated sludge systems. Two types are the most common: the completely mixed lagoon (also called completely suspended) in which the concentration of solids and dissolved oxygen are maintained fairly uniform and neither the incoming solids nor the biomass of microorganisms settle, and the facultative (aerobic-anaerobic or partially suspended) lagoons. In the facultative lagoons, the power input is reduced causing accumulation of solids in the bottom which undergo anaerobic decomposition, while the upper portions are maintained aerobic. The main operational difference between these lagoons is the power input, which is in the order of 2.5-6 Watts per cubic meter (W/m^3) for aerobic lagoons while the requirements for facultative lagoons are of 0.8-1 W/m^3. Being open to the atmosphere, the lagoons are exposed to low temperatures which can cause reduced biological activity and eventually the formation of ice. This can be partially alleviated by increasing the depth of the basin. These units require a secondary sedimentation unit, which in some cases can be a shallow basin excavated in earth, or conventional settling tanks can be used.

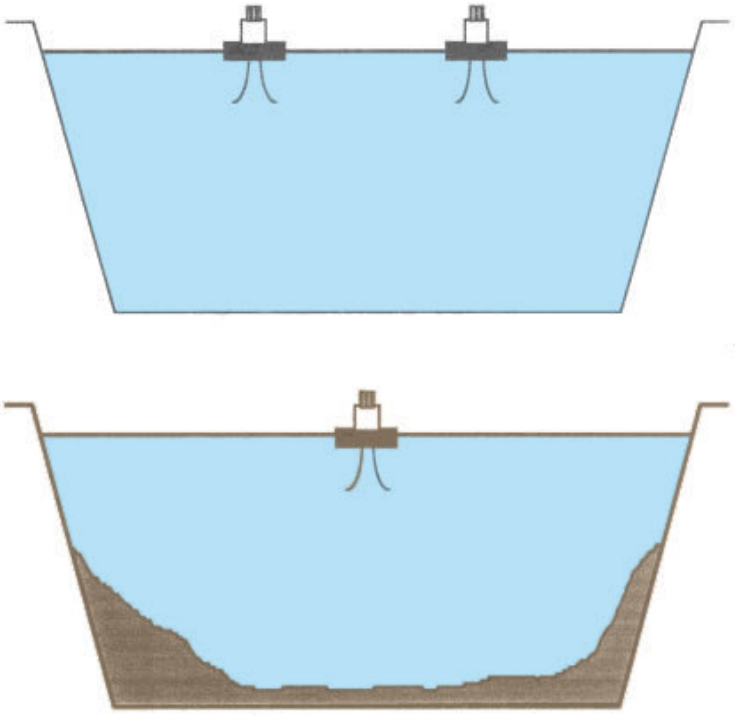


Diagram of aerobic (top) and facultative (bottom) aerated lagoons.

If excavated basins are used for settling, care should be taken to provide a residence time long enough for the solids to settle, and there should also be provision for the accumulation of sludge. There is a very high possibility of offensive odor development due to the decomposition of the settled sludge, and algae might develop in the upper layers contributing to an increased content of suspended solids in the effluent. Odors can be minimized by using minimum depths of up to 2 m, while algae production is reduced with liquid retention time of less than two days. The solids will also accumulate, all along the aeration basins in the facultative lagoons and even in comers, or between aeration units in the completely mixed lagoon. These accumulated solids will, on the whole, decompose in the bottom, but since there is always a non-biodegradable fraction, a permanent deposit will build up. Therefore, periodic removal of these accumulated solids becomes necessary. We will cover this in much more detail in a few more pages.

Nitrification

It was once thought that only two bacteria were involved in nitrification: Nitrosomonas europaea, which oxidizes ammonia to nitrite, and Nitrobacter winogradskyi, which oxidizes nitrite to nitrate. It is now known that at least 5 genera of bacteria oxidize ammonia and at least three genera of bacteria oxidize nitrite (Holt et al., 1994). Besides oxygen, these nitrifying bacteria require a neutral pH (7-8) and substantial alkalinity (these autotrophs use CO2 as a carbon source for growth). This indicates that complete nitrification would be expected at pond pH values between pH 7.0 and 8.5. Nitrification ceases at pH values above pH 9 and declines markedly at pH values below 7. This results from the growth inhibition of the nitrifying bacteria. Nitrification, however, is not a major pathway for nitrogen removal in lagoons. Nitrifying bacteria exists in low numbers in lagoons. They prefer attached growth systems and/or high MLSS sludge systems.

Anaerobic Bacteria
Anaerobic, heterotrophic bacteria that commonly occur in lagoons are involved in methane formation (acid-forming and methane bacteria) and in sulfate reduction (sulfate reducing bacteria). Anaerobic methane formation involves three different groups of anaerobic bacteria that function together to convert organic materials to methane via a three-step process. General anaerobic degraders - many genera of anaerobic bacteria hydrolyze proteins, fats, and poly saccharides present in wastewater to amino acids, short-chain peptides, fatty acids, glycerol, and mono- and di-saccharides. These have a wide environmental tolerance in pH and temperature.

Photosynthetic Organisms
Acid-forming bacteria - this diverse group of bacteria converts products from above under anaerobic conditions to simple alcohols and organic acids such as acetic, propionic, and butyric. These bacteria are hardy and occur over a wide pH and temperature range.

Methane forming bacteria - these bacteria convert formic acid, methanol, methylamine, and acetic acid under anaerobic conditions to methane. Methane is derived in part from these compounds and in part from CO_2 reduction. Methane bacteria are environmentally sensitive and have a narrow pH range of 6.5-7.5 and require temperatures > 14^{o} C.

Note that the products of the acid formers (principally acetic acid) become the substrate for the methane producers. A problem exists at times where the acid formers overproduce organic acids, lowering the pH below where the methane bacteria can function (a pH < 6.5). This can stop methane formation and lead to a buildup of sludge in a lagoon with a low pH. In an anaerobic fermenter, this is called a "stuck digester". Also, methane fermentation ceases at cold temperature, probably not occurring in most lagoons in the wintertime in cold climates. A number of anaerobic bacteria (14 genera reported to date (Bolt et al., 1994)) called sulfate reducing bacteria can use sulfate as an electron acceptor, reducing sulfate to hydrogen sulfide.

This occurs when BOD and sulfate are present and oxygen is absent. Sulfate reduction is a major cause of odors in ponds. Anaerobic, photosynthetic bacteria occur in all lagoons and are the predominant photo-synthetic organisms in anaerobic lagoons, The anaerobic sulfur bacteria, generally grouped into the red and green sulfur bacteria and represented by about 28 genera (Ehrlich, 1990), oxidize reduced sulfur compounds (H_2S) using light energy to produce sulfur and sulfate, Here, H_2S is used in place of H_2O as used by algae and green plants, producing $S0_4$- instead of O_2. All are either strict anaerobes or microaerophilic. Most common are Chromatium, Thiocystis, and Thiopedia, which can grow in profusion and give a lagoon a pink or red color. Finding them is most often an indication of organic overloading and anaerobic conditions in an intended aerobic system.

Conversion of odorous sulfides to sulfur and sulfate by these sulfur bacteria is a significant odor control mechanism in facultative and anaerobic lagoons, and can be desirable.

Major Algae Groups

Blue-green algae are the slimy stuff. Its cells lack nuclei and its pigment is scattered. Blue-green algae are not actually algae, they are bacteria.

Green algae cells have nuclei and the pigment is distinct. Green algae are the most common algae in ponds and can be multicellular.

Euglenoids are green or brown and swim with their flagellum, too. They are easy to spot because of their red eye. Euglenoids are microscopic and single celled.

Dinoflagellates have a flagella and can swim in open waters. They are microscopic and single celled.

Diatoms look like two shells that fit together. They are microscopic and single celled.

Algae

Algae are aerobic organisms that are photosynthetic and grow with simple inorganic compounds CO_2, NH_3, NO_3, and PO_4 using light as an energy source. (**Note that algae produce oxygen during the daylight hours and consume oxygen at night.)

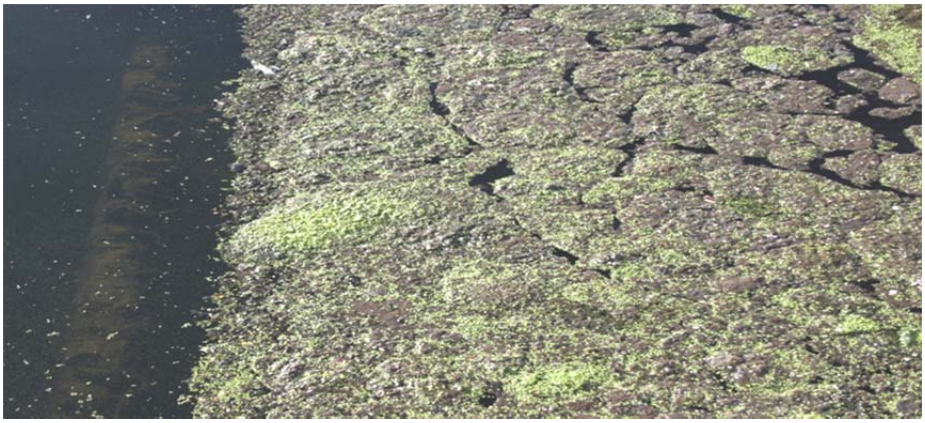
Algae are desirable in lagoons as they generate oxygen needed by bacteria for waste stabilization. Three major groups occur in lagoons, based on their chlorophyll type: brown algae (diatoms), green algae, and red algae. The predominant algal species at any given time is dependent on growth conditions, particularly temperature, organic loading, oxygen status, nutrient availability, and predation pressures. A fourth type of "algae" common in lagoons is the cyanobacteria or blue-green bacteria.

These organisms grow much as the true algae, with the exception that most species can fix atmospheric nitrogen. Blue-green bacteria often bloom in lagoons and some species produce odorous and toxic by-products.

Blue-Green Bacteria
Blue-green bacteria appear to be favored by poor growth conditions including high temperature, low light, low nutrient availability (many fix nitrogen) and high predation pressure. Common blue-green bacteria in waste treatment systems include **Aphanothece, Microcystis, Oscillatoria and Anabaena**.

Algae can bloom in lagoons at any time of the year (even under the ice); however, a succession of algae types occurs over the season. There is also a shift in the algal species present in a lagoon through the season, caused by temperature and rotifer and Daphnia predation. Diatoms usually predominate in the wintertime at temperatures <60°F. In the early spring, when predation is low and lagoon temperatures increase above 60°F, green algae such as Chlorella, Chlamydomonas, and Euglena often predominate in waste treatment lagoons. The predominant green algae change to species with spikes or horns such as Scenesdesmus, Micractinium, and Ankistrodesmus later in the season when Rotifers and Daphnia are active
(these species survive predation better).

Algae grow at warmer temperatures, longer detention time, and when inorganic minerals needed for growth are in excess. Alkalinity (inorganic carbon) is the only nutrient likely to be limiting for algal growth in lagoons. Substantial sludge accumulation in a lagoon may become soluble upon warming in the spring, releasing algal growth nutrients and causing an algal bloom. Sludge resolution of nutrients is a major cause of high algal growth in a lagoon, requiring
sludge removal from the lagoon for correction.



Algae on the Secondary clarifier, this is not a good sign.

Treatment Lagoon

The pH at a treatment lagoon is determined by the various chemical species of alkalinity that are present. The main species present are carbon dioxide (CO_2, bicarbonate ion (HCO_3), and carbonate ion (CO_2^3). Alkalinity and pH can affect which species will be present. High amounts of CO_2 yield a low lagoon pH, while high amounts of CO_2^3 yield a high lagoon pH.

Bacterial growth on BOD releases CO_2 which subsequently dissolves in water to yield carbonic acid (H_2CO_3). This rapidly dissociates to bicarbonate ion, increasing the lagoon alkalinity. Bacterial oxidation of BOD causes a decrease in lagoon pH due to CO_2 release.

Algal growth in lagoons has the opposite effect on lagoon pH, raising the pH due to algal use for growth of inorganic carbon (CO_2 and HCO_3). Algal growth reduces the lagoon alkalinity which may cause the pH to increase if the lagoon alkalinity (pH buffer capacity) is low. Algae can grow to such an extent in lagoons (a bloom) that they consume all of the CO_2 and HCO_3 present for photosynthesis, leaving only carbonate (CO_2^3) as the pH buffering species. This causes the pH of the lagoon to become alkaline. pH values of 9.5 or greater are common in lagoons during algal blooms, which can lead to lagoon effluent pH violations (in most states this is pH = 9). It should be noted that an increase in the lagoon pH caused by algal growth can be beneficial. Natural disinfection of pathogens is enhanced at higher pH.

Phosphorus removal by natural chemical precipitation is greatly enhanced at pH values greater than pH = 8.5. In addition, ammonia stripping to the atmosphere is enhanced at higher pH values (NH_3 is strippable, not NH_4+).

Protozoans and Microinvertebrates
Many higher life forms (animals) develop in lagoons. These include protozoans and microinvertebrates such as rotifers, daphnia, annelids, chironomids (midge larvae), and mosquito larvae (often termed the zooplankton). These organisms play a role in waste purification by feeding on bacteria and algae and promoting flocculation and settling of particulate material.

Protozoans are the most common higher life forms in lagoons with about 250 species identified in lagoons to date (Curds, 1992). Rotifers and daphnia are particularly important in controlling algal overgrowth and these often "bloom" when algal concentrations are high.

These microinvertebrates are relatively slow growing and generally only occur in systems with a detention time of >10 days. Mosquitoes grow in lagoons where shoreline vegetation is not removed, possibly causing a nuisance and public health problem.

Culex tarsalis, the vector of Western Equine Encephalitis in the western U.S., grows well in wastewater lagoons (USEPA, 1983). The requirement for a minimum lagoon bank slope and removal of shoreline vegetation by most regulatory agencies is based on the public health need to reduce mosquito vectors.

Activated Sludge Methods

We have some wastewater treatment plants that grow the microorganisms (Bugs) in large tanks. To have enough oxygen in the tanks we add oxygen by blowing air into the tank full of waste-water and microorganisms. The air is bubbled in the water and mixes *"the bugs,"* food and oxygen together. When we treat wastewater this way, we call it the activated sludge method. With all of this food and air, the microbes grow and multiply very rapidly.

Pretty soon the population of bugs gets too large and some of them need to be removed to make room for new bugs to grow. We remove the excess bugs by sedimentation in the same kind of tanks used for primary treatment. In the tank, the bugs sink to the bottom and we remove them. The settled bugs are also called waste activated sludge. The waste sludge is treated separately, and the remaining wastewater is now much cleaner. In fact, after primary and secondary treatment, about 85% or more of all pollutants in the wastewater has been removed and it goes on to Disinfection. These systems originated in England in the early 1900's and earned their name because a sludge (mass of microbes) is produced which aerobically degrades and stabilizes the organic load of a wastewater. Below diagram shows the layout of a typical activated sludge system.

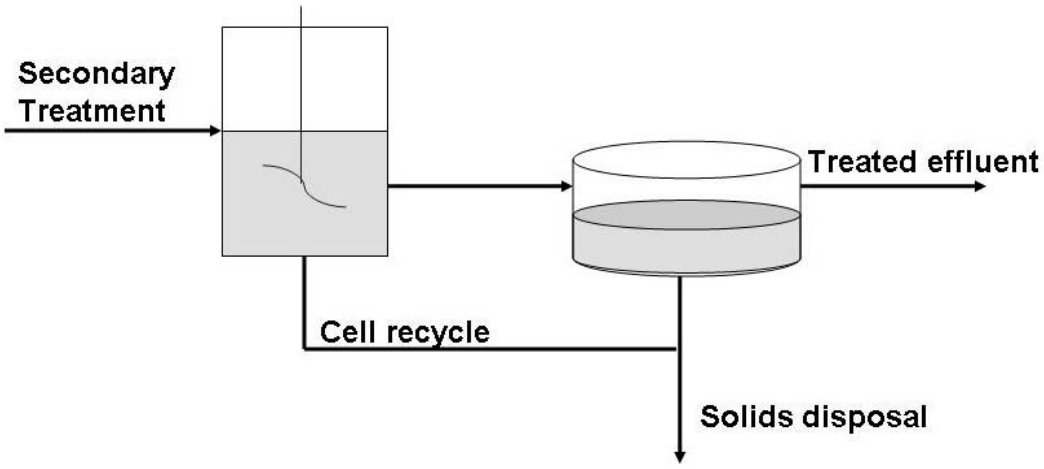


Diagram of a simple activated sludge system.

For larger systems, especially when high variability is expected, the design involves the use of multiple aeration tanks and multiple settling tanks. The number of units employed depends on the flow of wastewater being generated.

Organic Load

The organic load (generally coming from primary treatment operations such as settling, screening or flotation) enters the reactor where the active microbial population (activated sludge) is present. The reactor must be continuously aerated. The mixture then passes to a secondary settling tank where the cells are settled. The treated wastewater is generally discharged after disinfection while the settled biomass is recycled in part to the aeration basin. The cells must be recycled in order to maintain sufficient biomass to degrade the organic load as quickly as possible. The amount that is recirculated depends on the need to obtain a high degradation rate and on the need for the bacteria to flocculate properly so that the secondary settling separates the cells satisfactorily. As the cells are retained longer in the system, the flocculating characteristics of the cells improve since they start to produce extra cellular slime which favors flocculating.

Common Types
The most common types of activated sludge are the conventional and the continuous flow stiffed tank, in which the contents are completely mixed. In the conventional process, the wastewater is circulated along the aeration tank, with the flow being arranged by baffles in plug flow mode. The oxygen demand for this arrangement is maximum at the inlet as is the organic load concentration.

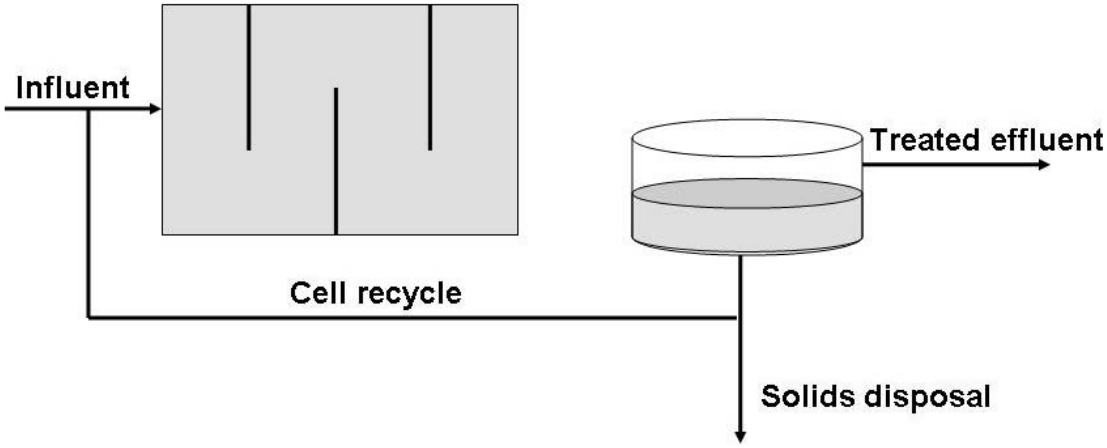


Diagram of a conventional activated sludge process.

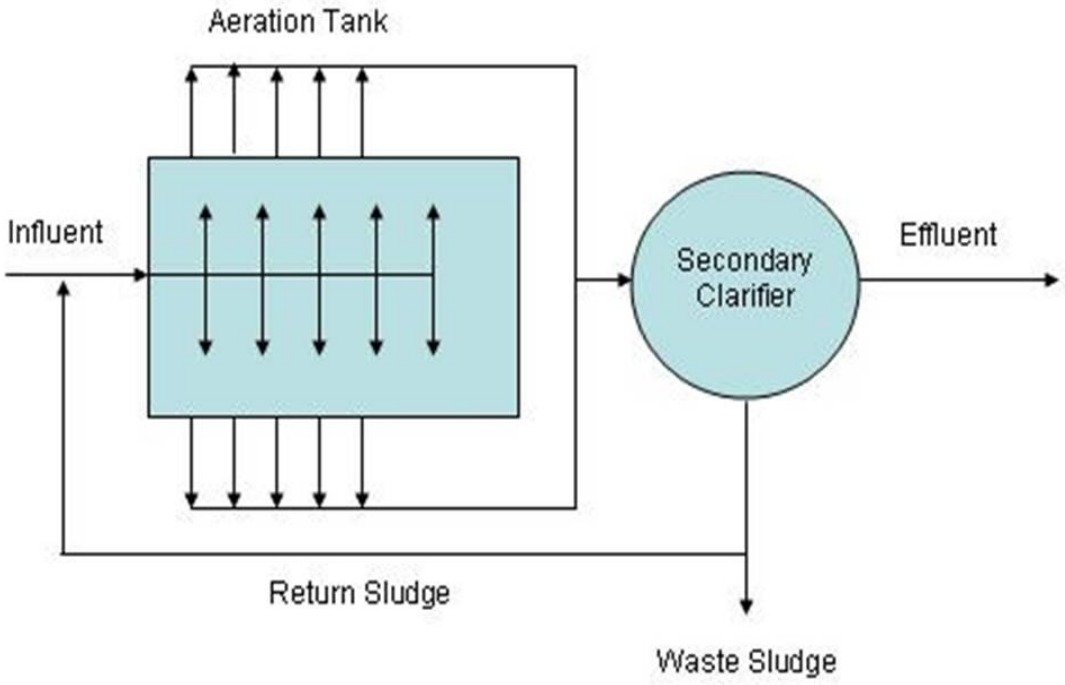
In the completely mixed process the inflow streams are usually introduced at several points to facilitate the homogeneity of the mixing; if the mixing is complete, the properties are constant throughout the reactor. This configuration is inherently more stable to perturbations because mixing causes the dilution of the incoming stream into the tank. In fisheries wastewaters the perturbations that may appear are peaks of concentration of organic load or flow peaks. The flow peaks can be damped in the primary treatment tanks. The conventional configurations would require less reactor volume if smooth plug flow could be assured, which usually does not occur.

Other versions of activated sludge systems (e.g., extended aeration, contact stabilization, step aeration and pure oxygen processes) are used in other kinds of wastewaters but are not known to be applied to treat fisheries wastewaters. They are discussed elsewhere (Metcalf and Eddy Inc., 1979; Eckenfelder, 1980). In all activated sludge systems, the cells are separated from the liquid and partially returned to the system to have a relatively high concentration of cells that degrade the organic load in a relatively short time.

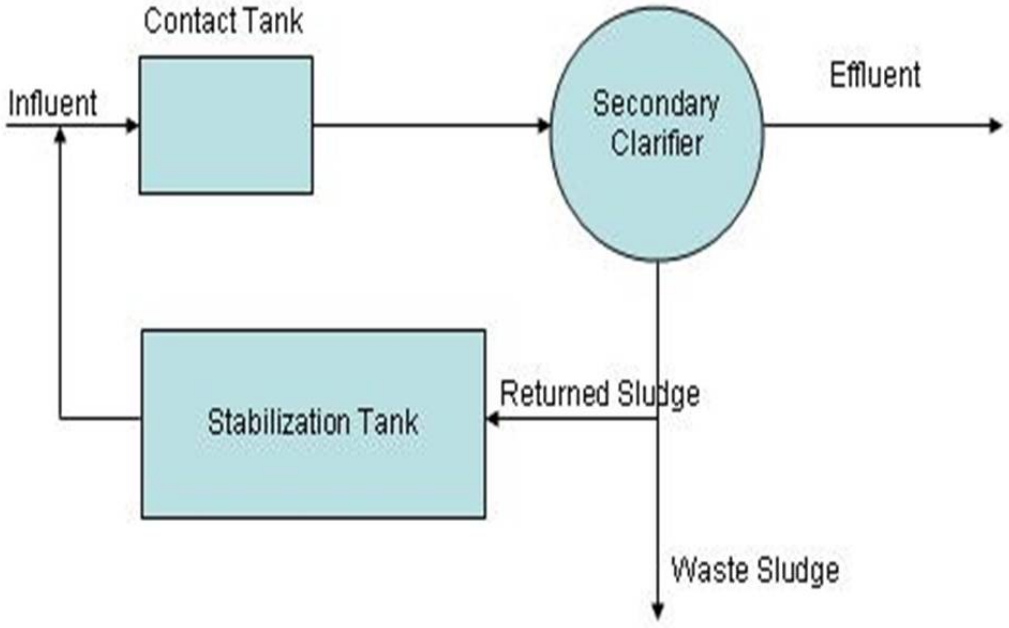
Therefore two different resident times are characteristic:
the hydraulic residence time ($\theta_{H)}$ given by the ratio of reactor volume (V) to flow of wastewater (Q):

$$\theta_{H} = V/Q$$

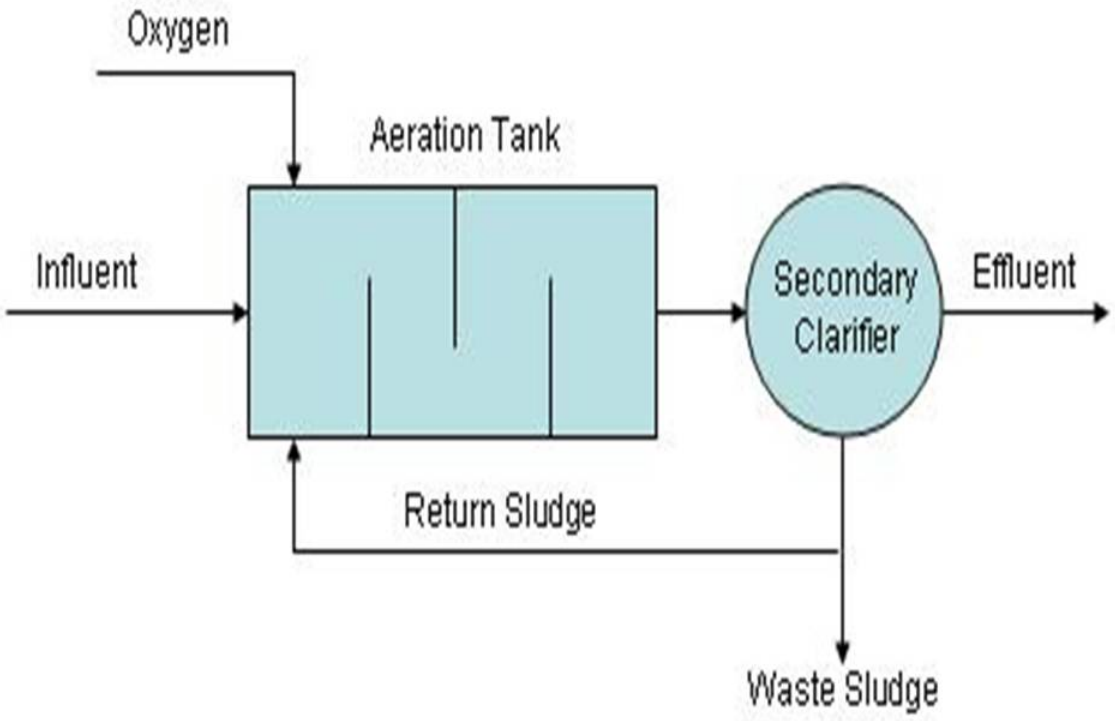
and the cell residence time (θc) given by the ratio of cells present in the reactor to the mass of cells wasted per day. Typical θ_H values are in the order of 3-6 hours, while θc fluctuates between 3 and 15 days. Such difference in resident times is obtained by discharging the clarified effluent but wasting only a small fraction of the sludge. This in turn can be accomplished by discarding a portion of the sludge from the settling tank or by wasting a fraction of the outlet of the reactor before entering the settling tank. In activated sludge systems, organic load removals of 85-95% are the most common. A key factor in the success of these systems is its proper operation, which requires trained manpower. Although used by some large fisheries which operate on a year-round basis, activated sludge may not prove to be economical or feasible for small seafood processors who operate seasonally because of the need to have a fairly constant supply of wastewater to maintain the micro-organisms.



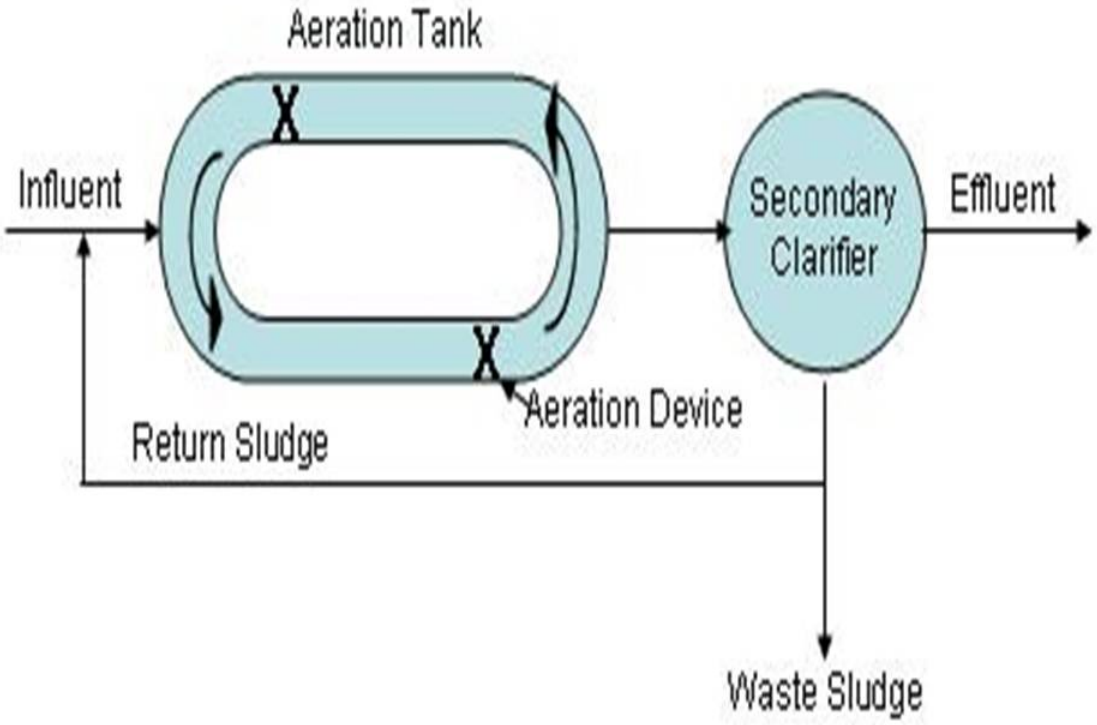
Complete Mix Activated Sludge Process



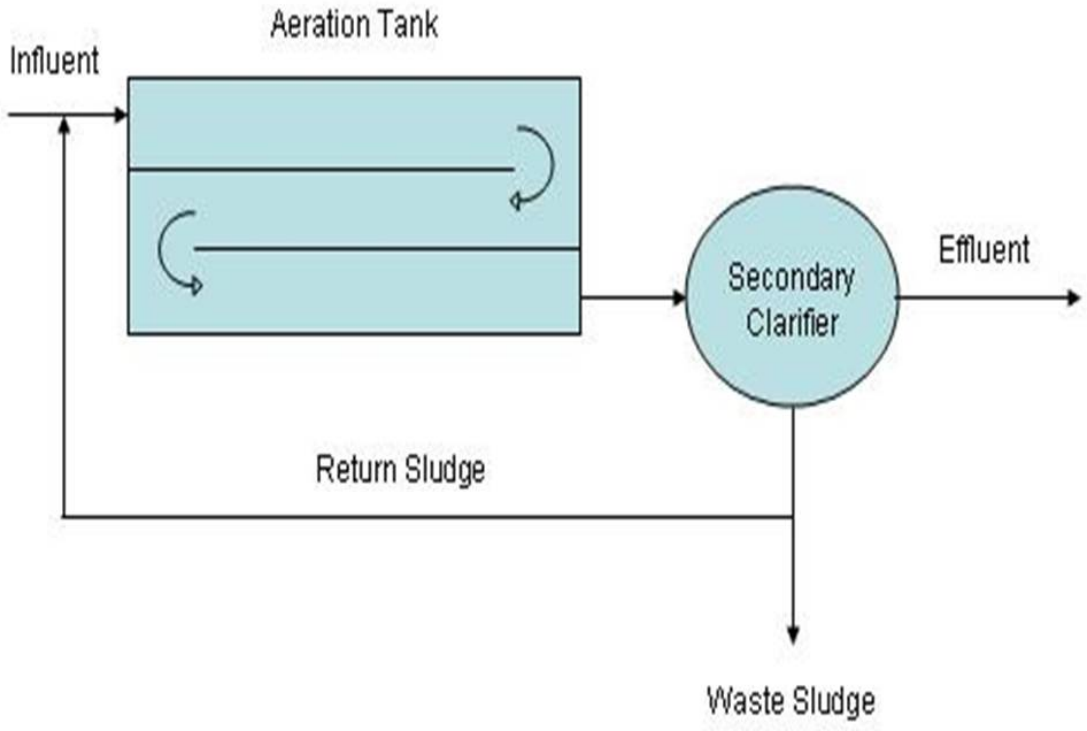
Contact Stabilization Activated Sludge



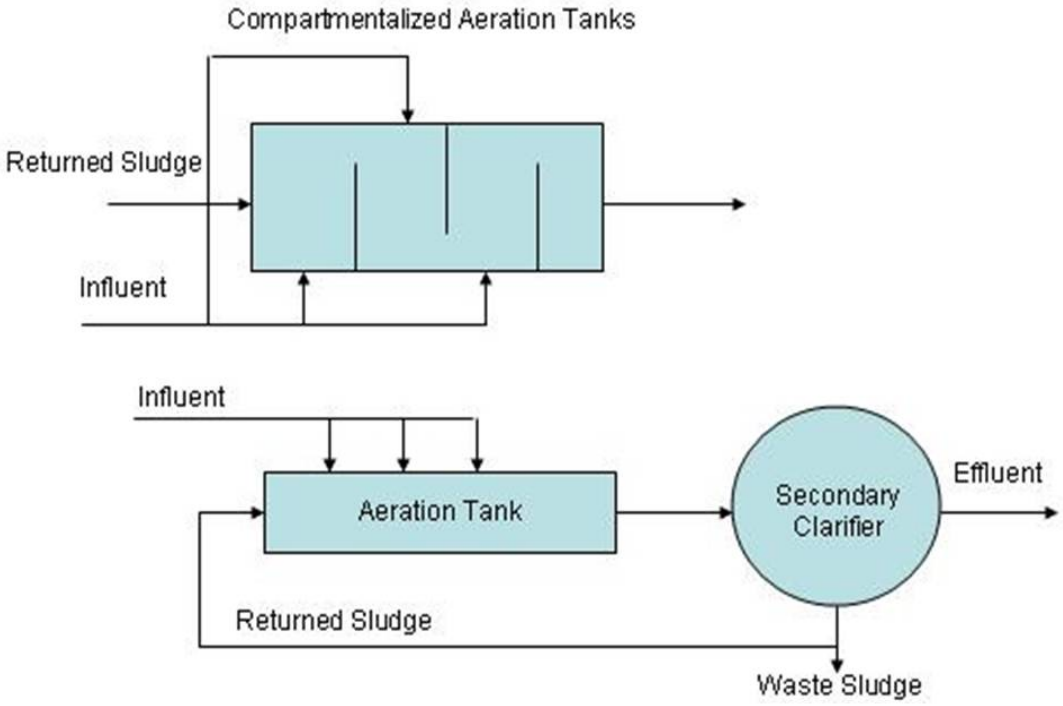
High Purity Oxygen Activated Sludge



Oxidation Ditch Activated Sludge Process



Plug Flow Activated Sludge Process



Step Feed Activated Sludge Process

Rectangular Clarifiers, notice the weirs are covered and protected from Sun light, the Sun helps the algae to grow on the weirs.

Key Activated Sludge Word

Amine: A functional group consisting of "-NH_2."

Amino acid: A functional group consisting of a carbon with a carboxylic acid, "-COOH" and an amine, "-NH_2." These compounds are the building blocks for proteins.

Anabolism: Biosynthesis, the production of new cellular materials from other organic or inorganic chemicals.

Anaerobes: A group of organisms that do not require *molecular* oxygen. These organisms, as well as all known life forms, require oxygen. These organisms obtain their oxygen from inorganic ions such as nitrate or sulfate or from protein.

Anaerobic process: A process that only occurs in the absence of molecular oxygen.

Anoxic process: A process that occurs only at very low levels of molecular oxygen or in the absence of molecular oxygen.

Biochemical oxygen demand (BOD): The amount of oxygen required to oxidize any organic matter present in a water during a specified period of time, usually 5 days. It is an indirect measure of the amount of organic matter present in a water.

Carbonaceous biochemical oxygen demand (CBOD): The amount of oxygen required to oxidize any carbon containing matter present in a water.

Chemical oxygen demand (COD): The amount of oxygen required to oxidize any organic matter in the water using harsh chemical conditions.

Decomposers: Organisms that utilize energy from wastes or dead organisms. Decomposers complete the cycle by returning nutrients to the soil or water and carbon dioxide to the air or water.

Denitrification: The anoxic biological conversion of nitrate to nitrogen gas. It occurs naturally in surface waters low in oxygen, and it can be engineered in wastewater treatment systems.

Deoxygenation: The consumption of oxygen by the different aquatic organisms as they oxidize materials in the aquatic environment.

Facultative: A group of microorganisms which prefer or preferentially use molecular oxygen when available, but are capable of using other pathways for energy and synthesis if molecular oxygen is not available.

F/M Ratio: Another method for control wasting is to maintain a constant food-to-microorganism (F:M or F/M) ratio. With this method, the operator will try to increase or decrease the MLVSS to match an increase or decrease in the BOD entering the plant. Most plants will operate best at a specific F/M ratio between 0.05 - 0.1. If the optimum F/M has been determined from experience and can be maintained, a good effluent may be produced with consistent plant operation. The F/M ratio is to be calculated at least weekly and related to the efficiency of treatment plant operation. An F/M ratio between 0.05 - 0.15 BOD/lb MLSS is usually considered acceptable for an extended aeration process.

Nitrification: The biological oxidation of ammonia and ammonium sequentially to nitrite and then nitrate. It occurs naturally in surface waters, and can be engineered in wastewater treatment systems. The purpose of nitrification in wastewater treatment systems is a reduction in the oxygen demand resulting from the ammonia.

Nitrogen fixation: The conversion of atmospheric (or dissolved) nitrogen gas into nitrate by microorganisms.

Nitrogenous oxygen demand (NOD): The amount of oxygen required to oxidize any ammonia present in a water.

NPDES: The National Pollutant Discharge Elimination System. The discharge criteria and permitting system established by the U.S. EPA as a result of the Clean Water Act and its subsequent amendments or the permit required by each discharger as a result of the Clean Water Act.

MCRT Mean Cell Residence Time: The average time a given unit of cell mass stays in the activated sludge biological reactor. It is typically calculated as the total mixed liquor suspended solids in the biological reactor divided by the combination of solids in the effluent and solids wasted.

Mixed liquor suspended solids (MLSS): The total suspended solids concentration in the activated sludge tank.

Mixed liquor volatile suspended solids (MLVSS): The volatile suspended solids concentration in the activated sludge tank.

Organic compound: Any compound containing carbon except for the carbonates (carbon dioxide, the carbonates and bicarbonates), the cyanides, and cyanates.

Organic nitrogen: Nitrogen contained as amines in organic compounds such as amino acids and proteins.

Oxidative phosphorylation: The synthesis of the energy storage compound adenosine triphosphate (ATP) from adenosine diphosphate (ADP) using a chemical substrate and molecular oxygen.

Secondary treatment: In wastewater treatment, the conversion of the suspended, colloidal and dissolved organics remaining after primary treatment into a microbial mass which is then removed in a second sedimentation process. Secondary treatment includes both the biological process and the associated sedimentation process.

Sludge: A mixture of solid waste material and water. Sludges result from the concentration of contaminants in water and wastewater treatment processes. Typical wastewater sludges contain from 0.5 to 10 percent solid matter. Typical water treatment sludges contain 8 to 10 percent solids.

Thiols: Organic compounds which contain the "-SH" functional group. Also called mercaptans.

Total dissolved solids: (TDS) Is the amount of dissolved matter in a water.

Total solids: (TS) Is the amount of organic and inorganic matter that is contained in a water.

Total suspended solids: (TSS) Is the amount of suspended (filterable) matter in a water.

Ultimate biochemical oxygen demand (BOD_u): The total amount of oxygen required to oxidize any organic matter present in a water, i.e. after an extended period, such as 20 or 30 days.

Virus: A submicroscopic genetic constituent that can alternate between two distinct phases. As a virus particle, or virion, it is DNA or RNA enveloped in an organic capsule. As an intracellular virus, it is viral DNA or RNA inserted into the host organisms DNA or RNA.

Volatile: A material that will vaporize easily.

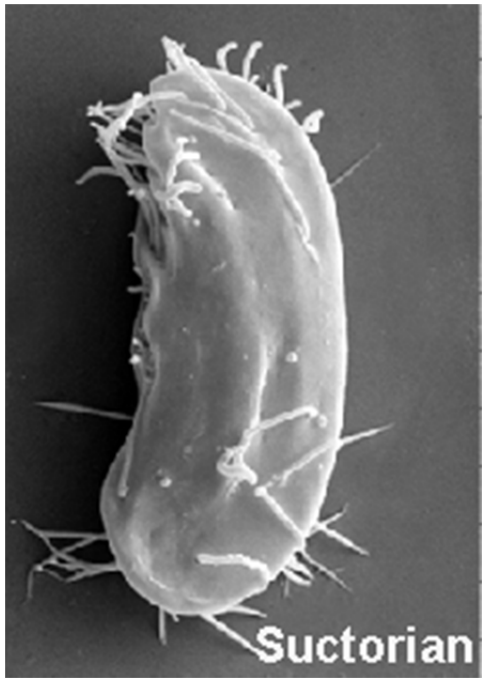
Volatile solids (VS) is the amount of matter which volatilizes (or burns) when a water sample is heated to 550^{o}C.

Gravity belt thickeners are often used to remove excess water from sludge.

Dry polymer is being added and used for sludge thickening.

Bugs or MOs

Four groups of bugs do most of the "eating" in the activated sludge process. The first group is the bacteria which eat the dissolved organic compounds. The second and third groups of bugs are microorganisms known as the free-swimming and stalked ciliates. These larger bugs eat the bacteria and are heavy enough to settle by gravity. The fourth group is a microorganism, known as Suctoria, which feeds on the larger bugs and assists with settling.



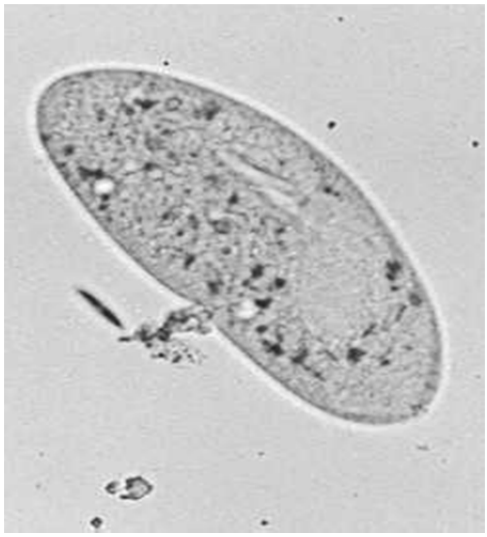
The interesting thing about the bacteria that eat the dissolved organics is they have no mouths. The bacteria have an interesting property, their "fat reserves" are stored on the outside of their bodies. This fat layer is sticky and is what the organics adhere to.

Once the bacteria have "contacted" their food, they start the digestion process. A chemical enzyme is sent out through the cell wall to break up the organic compounds. This enzyme, known as hydrolytic enzyme, breaks the organic molecules into small units which are able to pass through the cell wall of the bacteria.

In wastewater treatment, this process of using bacteria-eating bugs in the presence of oxygen to reduce the organics in water is called activated sludge. The first step in the process, the contact of the bacteria with the organic compounds, takes about 20 minutes. The second step is the breaking up, ingestion and digestion processes, which takes four to 24 hours.

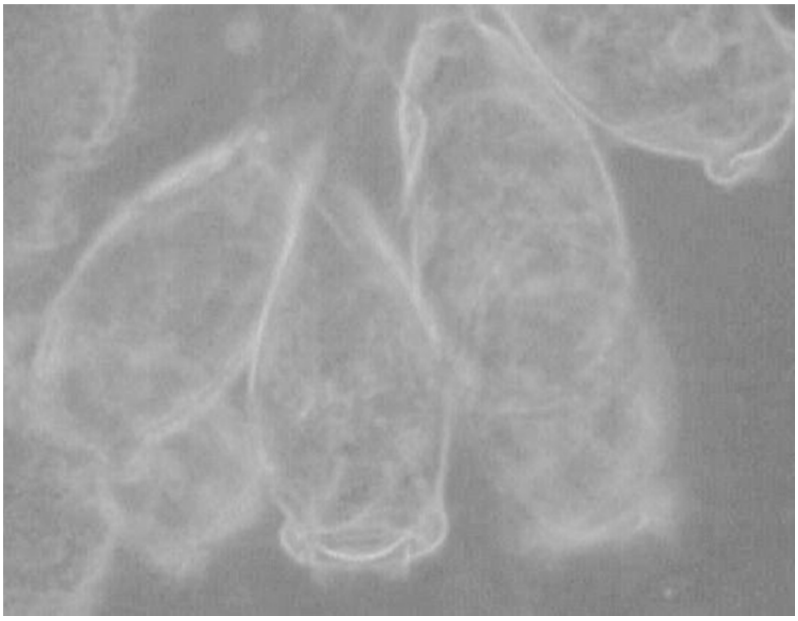
The fat storage property of the bacteria is also an asset in settling. As the bugs "bump" into each other, the fat on each of them sticks together and causes flocculation of the non-organic solids and biomass. From the aeration tank, the wastewater, now called mixed liquor, flows to a secondary clarification basin to allow the flocculated biomass of solids to settle out of the water. The solids biomass, which is the activated sludge, contains millions of bacteria and other microorganisms, is used again by returning it to the influent of the aeration tank for mixing with the primary effluent and ample amounts of air.

Paramecium sp.



Paramecium is a medium to large size (100-300 µm) swimming ciliate, commonly observed in activated sludge, sometimes in abundant numbers. The body is either foot-shaped or cigar-shaped, and somewhat flexible. Paramecium is uniformly ciliated over the entire body surface with longer cilia tufts at the rear of the cell. Paramecium swims with a smooth gliding motion. It may also be seen paired up with another Paramecium which makes a good diagnostic key. The cell has either one or two large water cavities which are also identification tools. This swimmer moves freely in the water column as it engulfs suspended bacteria. It has a large feeding groove used to trap bacteria and form the food cavities that move throughout the body as digestion occurs. Paramecium is described as a filter-feeding ciliate because its cilia move and filter bacteria from the water.

Vorticella sp.

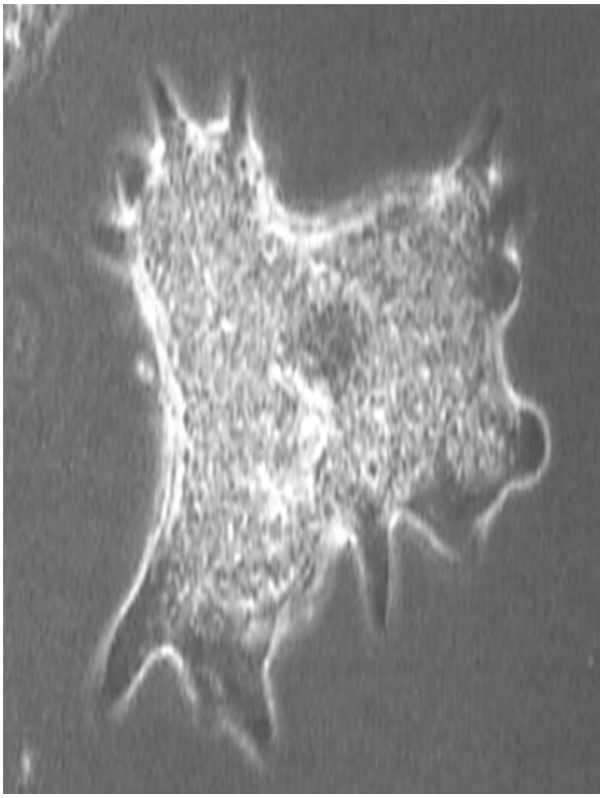


Vorticella is a stalked ciliate. There are at least a dozen species found in activated sludge ranging in length from about 30 to 150 µm. These organisms are oval to round shaped, have a contractile stalk, a domed feeding zone, and a water vacuole located near the terminal end of the feeding cavity. One organism is found on each stalk except during cell division. After reproducing, the offspring develops a band of swimming cilia and goes off to form its own stalk. The evicted organism is called a "***swarmer***."

Vorticella feeds by producing a vortex with its feeding cilia. The vortex draws bacteria into its gullet. Vorticella's principal food source is suspended bacteria. The contracting stalk provides some mobility to help the organism capture bacteria and avoid predators.

The stalk resembles a coiled spring after its rapid contraction. Indicator: If treatment conditions are bad, for example low DO or toxicity, Vorticella will leave their stalks. Therefore, a bunch of empty stalks indicates poor conditions in an activated sludge system. Vorticella sp. are present when the plant effluent quality is high.

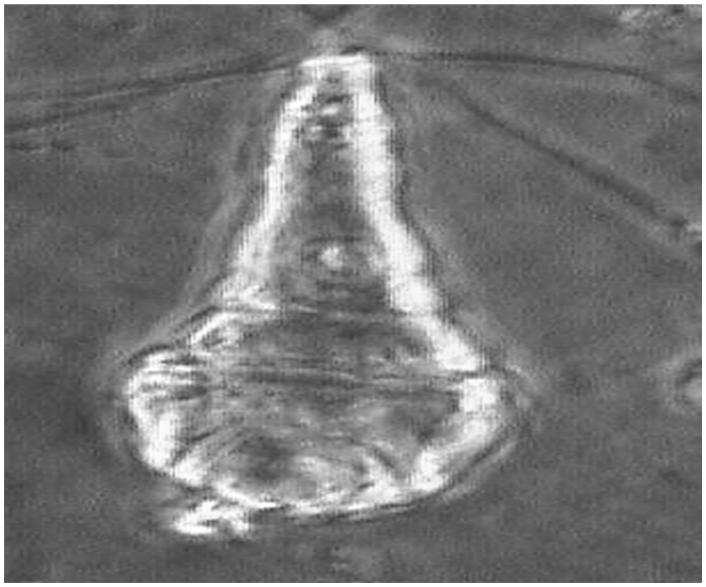
Euglypha sp.



Euglypha (70-100 µm) is a shelled (testate) amoeba. Amoebas have jelly-like bodies. Motion occurs by extending a portion of the body (pseudopodia) outward. Shelled amoebas have a rigid covering which is either secreted or built from sand grains or other extraneous materials. The secreted shell of this Euglypha sp. consists of about 150 oval plates. Its spines project backward from the lower half of the shell. Euglypha spines may be single or in groups of two or three. The shell has an opening surrounded by 8-11 plates that resemble shark teeth under very high magnification.

The shell of Euglypha is often transparent, allowing the hyaline (watery) body to be seen inside the shell. The pseudopodia extend outward in long, thin, rays when feeding or moving. Euglypha primarily eats bacteria. Indicator: Shelled amoebas are common in soil, treatment plants, and stream bottoms where decaying organic matter is present. They adapt to a wide range of conditions and therefore are not good indicator organisms.

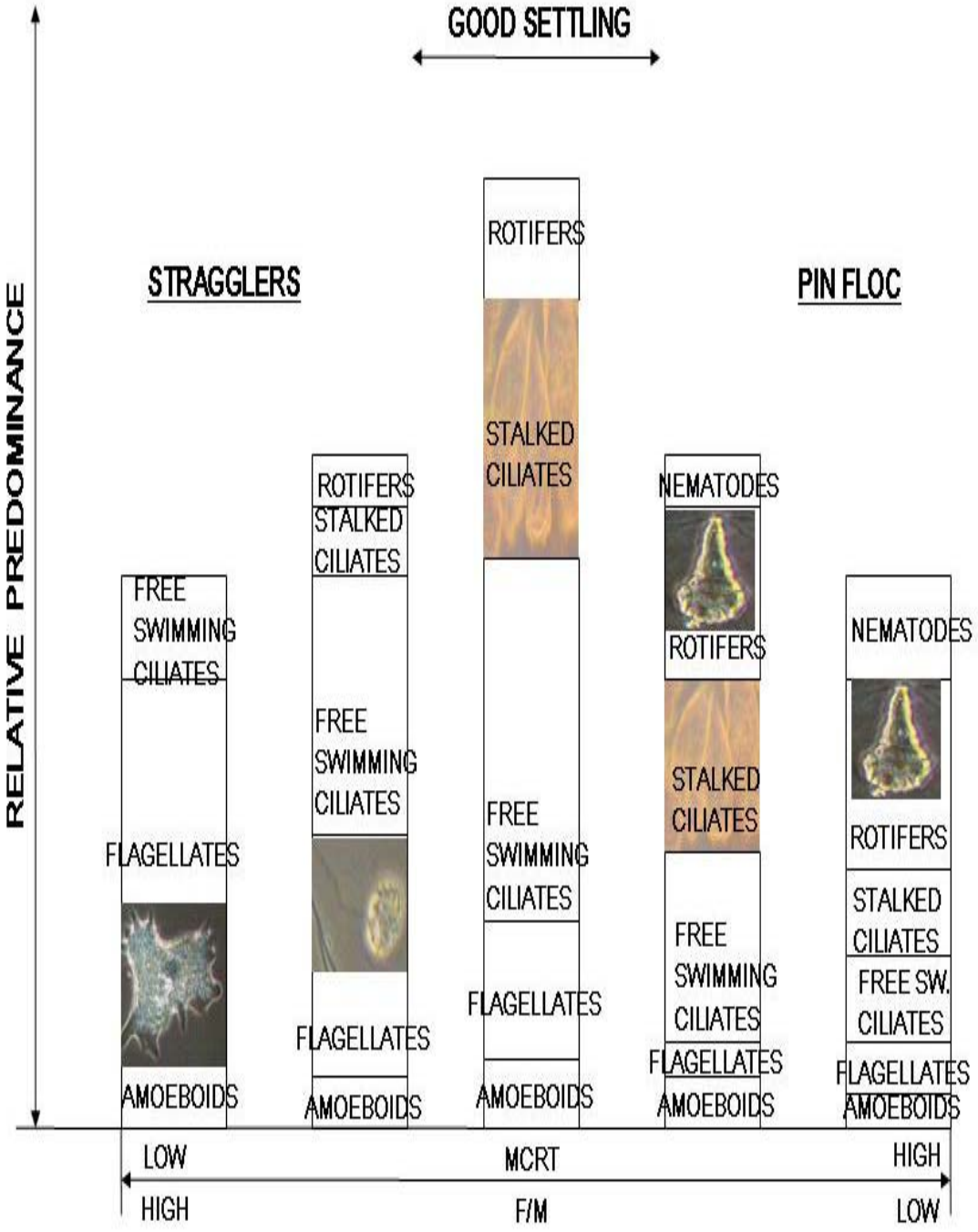
Euchlanis sp.



This microscopic animal is a typical rotifer. Euchlanis is a swimmer, using its foot and cilia for locomotion. In common with other rotifers, it has a head rimmed with cilia, a transparent body, and a foot with two strong swimming toes.

The head area, called the "corona," has cilia that beat rhythmically, producing a strong current for feeding or swimming. Euchlanis is an omnivore, meaning that its varied diet includes detritus, bacteria, and small protozoa. Euchlanis has a glassy shell secreted by its outer skin. The transparent body reveals the brain, stomach, intestines, bladder, and reproductive organs.

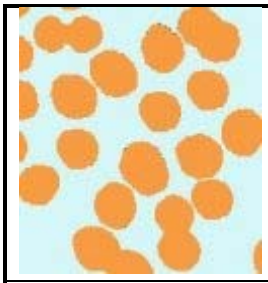
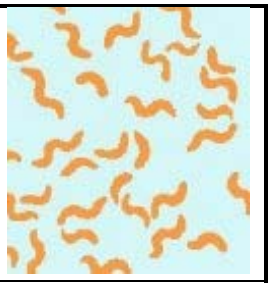
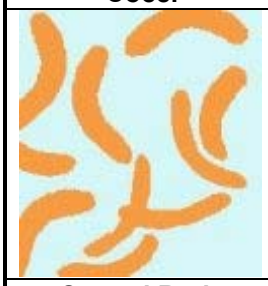
A characteristic of rotifers is their mastax, which is a jaw-like device that grinds food as it enters the stomach. At times the action of the mastax resembles the pulsing action of a heart. Rotifers, however, have no circulatory system. Indicator: Euchlanis is commonly found in activated sludge when effluent quality is good. It requires a continual supply of dissolved oxygen, evidence that aerobic conditions have been sustained.



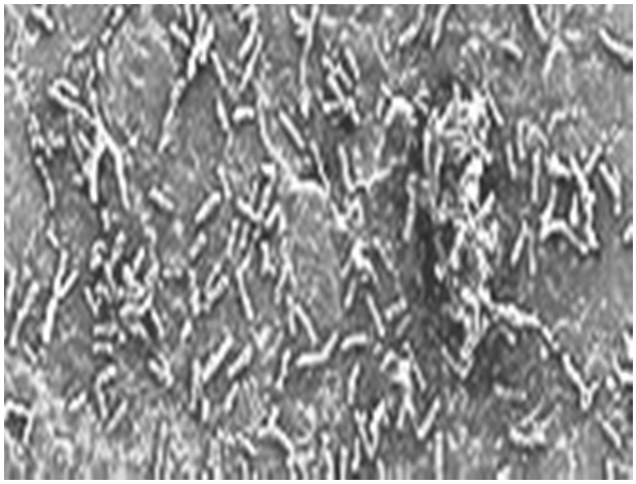
Wastewater Treatment Microlife

Bacteria Section

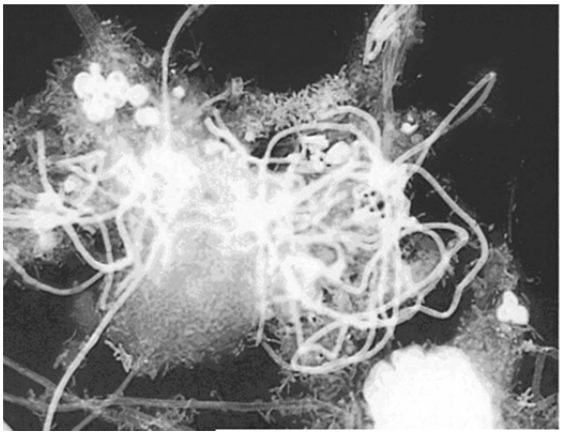
Bacteria are one of the most ancient of living things and scientists believe they have been on this planet for nearly 4,000 million years. During this time they have acquired lots of fascinating and different ways of living. They also come in a variety of shapes. The simplest shape is a round sphere or ball. Bacteria formed like this are called cocci (singular coccus). The next simplest shape is cylindrical. Cylindrical bacteria are called rods (singular rod). Some bacteria are basically rods but instead of being straight they are twisted, bent or curved, sometimes in a spiral. These bacteria are called spirilla (singular spirillum). Spirochaetes are tightly coiled up bacteria.

			
Cocci	**Rods**	**Ovoids**	**Spira**
			
Curved Rods	**Curved Rods**	**Spirochaetes**	**Filamentous**

Bacteria are friendly creatures; you never find one bacteria on its own. They tend to live together in clumps, chains or planes. When they live in chains, one after the other, they are called filamentous bacteria - these often have long thin cells. When they tend to collect in a plane or a thin layer over the surface of an object, they are called a biofilm. Many bacteria exist as a biofilm and the study of biofilms is very important. Biofilm bacteria secrete sticky substances that form a sort of gel in which they live. The plaque on your teeth that causes tooth decay is a biofilm.



Filamentous Bacteria



Filamentous Bacteria are a type of bacteria that can be found in a wastewater treatment system.

They function similar to floc forming bacteria since they degrade BOD quite well. In small amounts, they are quite good to a biomass. They can add stability and a backbone to the floc structure that keeps the floc from breaking up or shearing due to turbulence from pumps, aeration or transfer of the water. In large amounts they can cause many problems. Filaments are bacteria and fungi that grow in long thread-like strands or colonies.

Site Specific Bacteria

Aeration and biofilm building are the key operational parameters that contribute to the efficient degradation of organic matter (BOD/COD removal). Over time, the application-specific bacteria become site-specific as the biofilm develops and matures and is even more efficient in treating the site-specific waste stream.

Facultative Bacteria

Most of the bacteria absorbing the organic material in a wastewater treatment system are facultative in nature. This means they are adaptable to survive and multiply in either anaerobic or aerobic conditions. The nature of individual bacteria is dependent upon the environment in which they live. Usually, facultative bacteria will be anaerobic unless there is some type of mechanical or biochemical process used to add oxygen to the wastewater. When bacteria are in the process of being transferred from one environment to another, the metamorphosis from anaerobic to aerobic state (and vice versa) takes place within a couple of hours.

Anaerobic Bacteria

Anaerobic bacteria live and reproduce in the absence of free oxygen. They utilize compounds such as sulfates and nitrates for energy and their metabolism is substantially reduced. In order to remove a given amount of organic material in an anaerobic treatment system, the organic material must be exposed to a significantly higher quantity of bacteria and/or detained for a much longer period of time. A typical use for anaerobic bacteria would be in a septic tank. The slower metabolism of the anaerobic bacteria dictates that the wastewater be held several days in order to achieve even a nominal 50% reduction in organic material. That is why septic tanks are always followed by some type of effluent treatment and disposal process. The advantage of using the anaerobic process is that electromechanical equipment is not required. Anaerobic bacteria release hydrogen sulfide as well as methane gas, both of which can create hazardous conditions. Even as the anaerobic action begins in the collection lines of a sewer system, deadly hydrogen sulfide or explosive methane gas can accumulate and be life threatening.

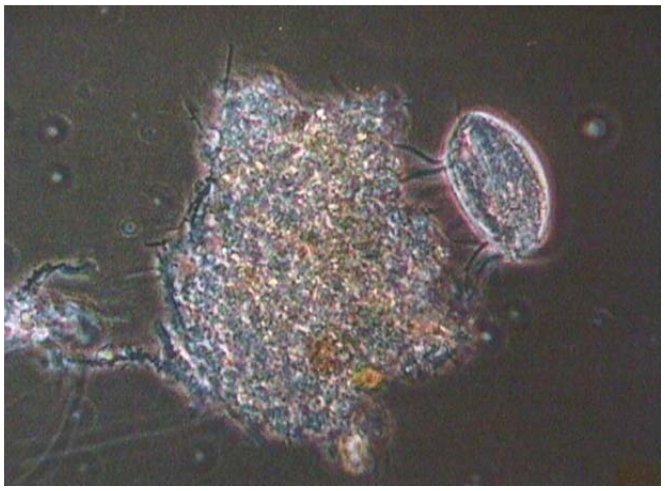
Aerobic Bacteria

Aerobic bacteria live and multiply in the presence of free oxygen. Facultative bacteria always achieve an aerobic state when oxygen is present. While the name "aerobic" implies breathing air, dissolved oxygen is the primary source of energy for aerobic bacteria. The metabolism of aerobes is much higher than for anaerobes. This increase means that 90% fewer organisms are needed compared to the anaerobic process, or that treatment is accomplished in 90% less time. This provides a number of advantages including a higher percentage of organic removal. The by-products of aerobic bacteria are carbon dioxide and water. Aerobic bacteria live in colonial structures called floc and are kept in suspension by the mechanical action used to introduce oxygen into the wastewater. This mechanical action exposes the floc to the organic material while treatment takes place. Following digestion, a gravity clarifier separates and settles out the floc. Because of the mechanical nature of the aerobic digestion process, maintenance and operator oversight are required.

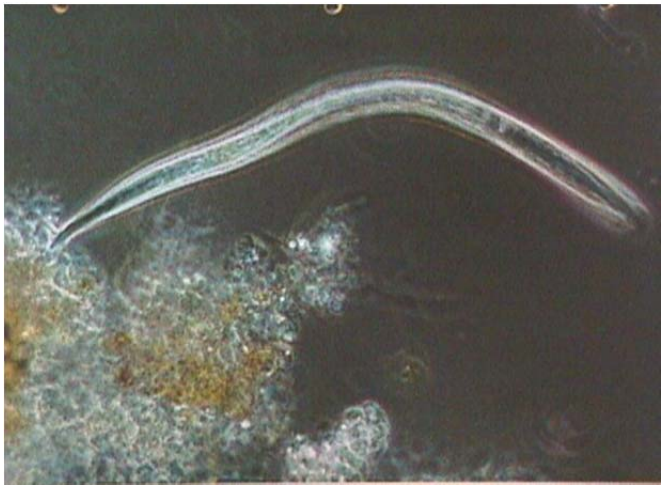
Protozoans and Metazoans

In a wastewater treatment system, the next higher life form above bacteria is protozoans. These single-celled animals perform three significant roles in the activated sludge process. These include floc formation, cropping of bacteria and the removal of suspended material. Protozoans are also indicators of biomass health and effluent quality. Because protozoans are much larger in size than individual bacteria, identification and characterization is readily performed. Metazoans are very similar to protozoans except that they are usually multi-celled animals. Macroinvertebrates, such as nematodes and rotifers, are typically found only in a well developed biomass.

The presence of protozoans and metazoans and the relative abundance of certain species can be a predictor of operational changes within a treatment plant. In this way, an operator is able to make adjustments and minimize negative operational effects simply by observing changes in the protozoan and metazoan population.



Aspidisca



Nematode

Dispersed Growth
Dispersed growth is material suspended within the activated sludge process that has not been adsorbed into the floc particles. This material consists of very small quantities of colloidal (too small to settle out) bacteria as well as organic and inorganic particulate material. While a small amount of dispersed growth between the floc particles is normal, excessive amounts can be carried through a secondary clarifier. When discharged from the treatment plant, dispersed growth results in higher effluent solids.

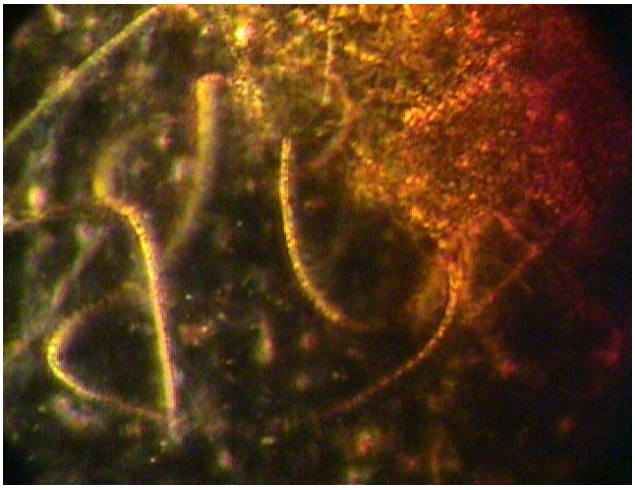
Taxonomy
Taxonomy is the science of categorizing life forms according to their characteristics. Eighteen different categories are used to define life forms from the broadest down to the most specific. They are: Kingdom, Phylum, Subphylum, Superclass, Class, Subclass, Cohort, Superorder, Order, Suborder, Superfamily, Family, Subfamily, Tribe, Genus, Subgenus, Species and Subspecies. Identifying the genus is usually specific enough to determine the role of the organisms found in a wastewater treatment system.

Process Indicators
Following taxonomic identification, enumeration and evaluation of the characteristics of the various organisms and structures present in a wastewater sample, the information can be used to draw conclusions regarding the treatment process.

Numerous industry references, such as ***WASTEWATER BIOLOGY: THE MICROLIFE*** by the Water Environment Federation, can be used to provide a comprehensive indication of the conditions within a treatment process. As an example, within most activated sludge processes, the shape of the floc particles can indicate certain environmental or operational conditions.

A spherical floc particle indicates immature floc, as would be found during start-up or a process recovery. A mature floc particle of irregular shape indicates the presence of a beneficial quantity of filamentous organisms and good quality effluent. An excess of dispersed growth could indicate a very young sludge, the presence of toxic material, excess mechanical aeration or an extended period of time at low dissolved oxygen levels.

Certain protozoans, such as amoebae and flagellates dominate during a system start-up. Free swimming ciliates are indicative of a sludge of intermediate health and an effluent of acceptable or satisfactory quality. A predominance of crawling ciliates, stalked ciliates and metazoans is an indicator of sludge with excellent health and an effluent of high quality.



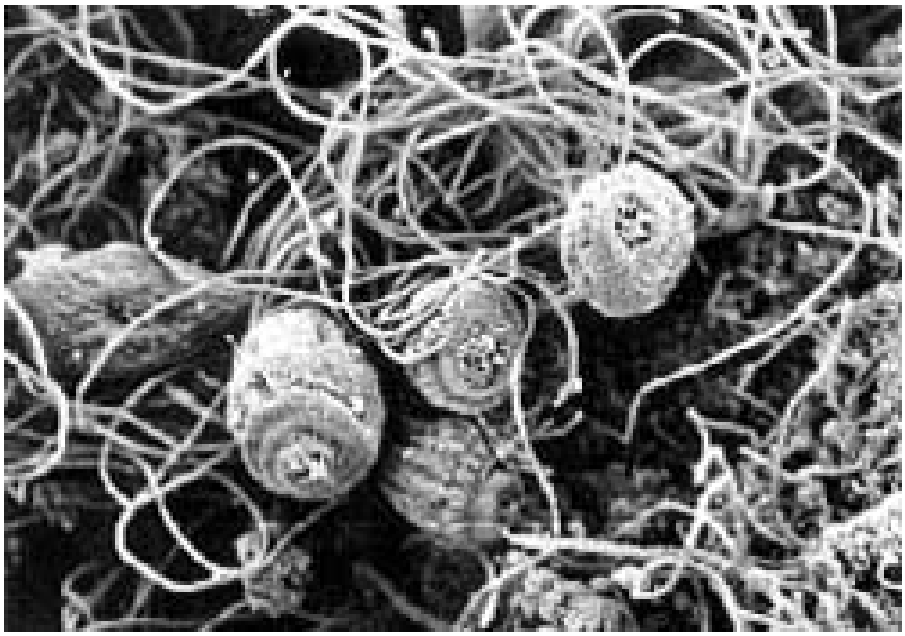
Filamentous Bacteria

Filamentous Bacteria have Positive aspects:

They are very good BOD removers
They add a backbone or rigid support network to the floc structure
Helps the floc structure filter out fine particulate matter that will improve clarifier efficiency.
They help the floc settle if in small amounts.
They reduce the amount of "pin" floc.

Filamentous Bacteria have Negative aspects:
They can interfere with separation and compaction of activated sludge and cause bulking when predominant.

Filamentous Bacteria
They can affect the sludge volume index (SVI).
They can cause poor settling if dominant.
They can fill up a clarifier and make it hard to settle, causing TSS carryover.
They can increase polymer consumption.
They can increase solids production and cause solids handling costs to increase significantly.



Filamentous bacteria floc (SEM) or Pin Floc.

Activated Sludge Aerobic Flocs

Aerobic flocs in a healthy state are referred to as activated sludge. While aerobic floc has a metabolic rate approximately 10 times higher than anaerobic sludge, it can be increased even further by exposing the bacteria to an abundance of oxygen. Compared to a septic tank, which takes several days to reduce the organic material, an activated sludge tank can reduce the same amount of organic material in approximately 4-6 hours. This allows a much higher degree of overall process efficiency. In most cases, treatment efficiencies and removal levels are so much improved that additional downstream treatment components are dramatically reduced or totally eliminated.

Problems may appear during the operation of activated sludge systems, including:

- High solids content in clarified effluent, which may be due to too high or too low solids retention time and to growth of filamentous microorganisms.
- Rising sludge, occurring when sludge that normally settles rises back to the surface after having settled. In most cases, this is caused by the denitrification process, where nitrate present in the effluent is reduced to nitrogen gas, which then becomes trapped in the sludge causing this to float. This problem can be reduced by decreasing the flow from the aeration basin to the settling tank or reducing the sludge resident time in the settler, either by increasing the rate of recycle to the aeration basin, increasing the rate of sludge collection from the bottom, or increasing the sludge wasting rate from the system.
- Bulking sludge, that which settles too slowly and is not compactable, caused by the predominance of filamentous organisms. This problem can be due to several factors of which the most common are nutrient balance, wide fluctuations in organic load, oxygen limitation (too low levels), and an improper sludge recycle rate.
- Insufficient reduction of organic load, probably caused by a low solids retention time, insufficient amount of nutrients such as P or N (rare in fisheries wastewaters), short-circuiting in the settling tank, poor mixing in the reactor and insufficient aeration or presence of toxic substances.
- Odors, caused by anaerobic conditions in the settling tanks or insufficient aeration in the reactor.

Filamentous Organisms

The majority of filamentous organisms are bacteria, although some of them are classified as algae, fungi or other life forms. There are a number of types of filamentous bacteria which proliferate in the activated sludge process. Filamentous organisms perform several different roles in the process, some of which are beneficial and some of which are detrimental. When filamentous organisms are in low concentrations in the process, they serve to strengthen the floc particles. This effect reduces the amount of shearing in the mechanical action of the aeration tank and allows the floc particles to increase in size.

Larger floc particles are more readily settled in a clarifier. Larger floc particles settling in the clarifier also tend to accumulate smaller particulates (surface adsorption) as they settle producing an even higher quality effluent. Conversely, if the filamentous organisms reach too high a concentration, they can extend dramatically from the floc particles and tie one floc particle to another (interfloc bridging) or even form a filamentous mat of extra-large size. Due to the increased surface area without a corresponding increase in mass, the activated sludge will not settle well. This results in less solids separation and may cause a washout of solid material from the system. In addition, air bubbles can become trapped in the mat and cause it to float, resulting in a floating scum mat.

Due to the high surface area of the filamentous bacteria, once they reach an excess concentration, they can absorb a higher percentage of the organic material and inhibit the growth of more desirable organisms.

Filamentous Bacteria Identification

Filamentous Identification should be used as a tool to monitor the health of the biomass when a filament problem is suspected. Filamentous Identification is used to determine the type of filaments present so that a cause can be found and corrections can be made to the system to alleviate future problems. All filamentous bacteria usually have a process control variation associated with the type of filament present that can be implemented to change the environment present and select out for floc forming bacteria instead. Killing the filaments with chlorine or peroxide will temporarily remove the filaments, but technically it is a band-aid. A process change must be made or the filaments will return with time eventually. Find out what filaments are present, find out the cause associated with them and make a process change for a lasting fix to the problems.

Filaments, their causes and suggested controls

Low DO Filaments	**Control**
Type 1701 S. natans	Adjust the aeration rates or F/M (based on aeration solids)
H. hydrossis	Long RAS lines or sludge held too long in the clarifier can sometimes cause the growth of low DO filaments even if the aeration
Waste with limited Nutrients	**Control**
Thiothrix I & II 021N and N. Limicola III	Nutrient addition BOD ratio of 100:5:1
Low F/M ratios	**Control**
0041, Nocardia	Use of selector, increase RAS
Type 1851, 0961, 0803, 0675	Increase WAS

Some filaments have more than one version of the filament species, with slightly different characteristics for identification.
N. Limicola I
N. Limicola II
N. Limicola III
Thiothrix I
Thiothrix II

Filamentous Identification
Filaments can be internal or external, and they can be free of the floc structures or found intertwined in the floc. Most labs think that filaments need to be extending from the floc in order to be a problem. This is not true. Internal filaments can cause more problems than external filaments. Think of internal filaments causing a structure like a sponge. It will retain water easily and be harder to dewater, will be hard to compress and will take up more space, thereby increasing solids handling costs.

Filaments present in the system do not always mean there is a problem. Some filaments are good if they form a strong backbone and add a rigid network to the floc. They help give the floc more structure and settle faster. Filaments are good BOD degraders also. They are only a problem when they become dominant. If filament abundance is in the abundant or excessive range, having a Filamentous Identification performed is recommended. When Gram and Neisser stains are performed for filamentous Identification, the types of filaments found present will be noted on the Floc Characterization sheet to the right of the filament section and will be noted on the Cover Sheet. A Filament Causes sheet, Filamentous Predominance sheet and corrective actions will be given and included with the report. A Filamentous Worksheet will be included. Individual sheets on the actual filaments present in the sample will be included with more information on that particular filament.

The activated sludge process was invented around 1914 and is today still the most commonly used biological wastewater treatment process. This widespread use is due to the fact that activated sludge can be a rather easy process to implement and one that can attain high treatment efficiency. That is to say, if it works! Activated sludge is susceptible to process disturbances making it a very problematic technology for many of its users. Problems arise most when the wastewater to be treated varies significantly in composition and/or flow.

Let's do a quick review of the Bugs…. We will go much more into detail later…

Nocardia amarae

Nocardia amarae, a common cause of disruptive foaming in waste treatment plants, is a slow growing, usually gram-positive, chemoautotrophic, filamentous, strict aerobe that produces the biosurfactant trehalose. Colonies can be brown, pink, orange, red, purple, gray or white, so color alone is not a key to identifying this species. N. amarae, member of the Actinomycetes family, is not motile, so it relies on movement of the water to carry it through the system. It produces catalase, urease and nitrate reductase enzymes, but not casease. The foam from Nocardia amarae is usually a viscous brown color unless algae are entrapped in it, in which case it appears green and brown.

Nostocoida limicola

Nostocoida limicola is yet another common cause of disruptive foaming in waste treatment plants, motile in its Hormogonia and sometimes Trichome phases. This oxygenic phototrophic species often forms a confluent gel encasing flattened discs or large sheets of cells, forming symbiotic relationships with other species. Staining gram-positive, Nostocoida produces round cells within tight coil formations. Nostocoida can also be dentified by their starburst effect formations using phase contrast microscopy at 400 to 1000x magnification. After chlorination, a few dead cells sticking out identify stress to this species.

Thiothrix

Thiothrix spp., the second most common cause of disruptive foaming in wastewater treatment plants appears as straight to slightly curved cells with rectangular shape form filaments up to 500 microns in length, in multicellular rigid filaments, staining gram-negative, with obligately aerobic respiration. Thiothrix are mixotrophic, using several small organic carbons and reduced inorganic sulfur sources for growth and energy. Thiothrix I is one of the largest filament found using phase contrast microscopy at 400 to 1000x magnification. Thiothrix II produces rectangular filaments up to 200 microns in length and is easily identified by their starburst effect formations using phase contrast microscopy at 400 to 1000x magnification.

Microthrix parvicella

Microthrix parvicella is another common cause of disruptive foaming in waste treatment plants, producing filaments up to 400 microns in length, easily visualized by phase contrast microscopy at 400x magnification. This species is usually found outside floc, tangling with structures in the system, but can also be found hanging out of the floc.

Sphaeroliticus natans

Sphaeroliticus natans is another filamentous species, and yet it is reputed to increase settleability by branching between flocs, increasing surface area. Cells are straight to slightly curved, up to 1000 microns in length and stain gram-negative. These large cells can be easily visualized by phase contrast microscopy at 100x magnification. Certain conditions favor the proliferation of filamentous species. A low F/M (food to mass) ratio favors filamentous organisms, because their higher ratio of surface area to volume provides them with a selective advantage for securing nutrients in nutrient limited environments. When a plant runs an extremely long sludge age, the slower growing filaments have a better chance to establish a strong colony. As a strict aerobe, high levels of oxygen are necessary to sustain this species. Mesophilic, Nocardia amarae thrives in temperatures from 17 to 37 deg. C.

The presence of high levels of fats, oils and greases or hydrocarbons and phenols, can encourage this species, particularly when insufficient levels of nitrogen and phosphorus are present to balance these carbon sources.

Filamentous Bacteria
A problem that often frustrates the performance of activated sludge is bulking sludge due to the growth of filamentous bacteria. Sludge bulking can often be solved by careful process modifications. However, different filamentous bacteria such as Microthrix, Sphaerotilus, Nostocoida, Thiothrix or ”Type 021N” and others cause bulking for very different reasons. Many filamentous species have not even been given a scientific name yet. Consequently, in order to make the right kind of process modification, knowledge to identify them and experience with the process ecology are required. The potential for instability with activated sludge is an acute problem when strict demands on treatment performance are in place.

PAX - finally, a Fix for Microthrix
If you ever experienced an overgrowth of Microthrix parvicella in your activated sludge plant, you will be aware that it can be very difficult to either eradicate or control. Microthrix is the most common cause of bulking and foaming in activated sludge plants (Rosetti et al. 2002), and it appears either essentially alone or in the company of other filaments.

Microthrix foams appear in many of the photographs of aeration basins and clarifiers I have collected all over the world, and many of the plant tours on the Internet show the same brown stable scums associated with this organism. Let's face it, Microthrix is just about everywhere.

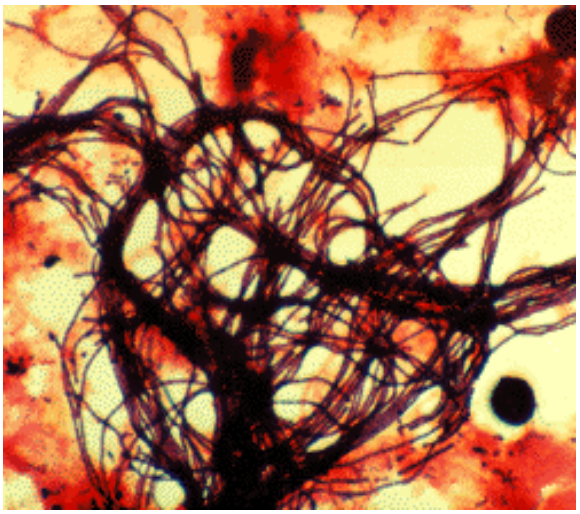


Figure 1.
A micrograph of Microthrix parvicella, gram stain x 1000

Microthrix is your enemy - Get to know it!

Microthrix fits into the filamentous bacterial classification of low F/M, which means that it tends to appear in plants with long sludge ages. Lackay *et al.* (1999) suggested that *M. parvicella* and its low F/M compatriots *Haliscomenobacter hydrossis*, and types 0092, 0041, 1851, 0803 were also encouraged to the point of maximum proliferation by alternating anoxic-aerobic conditions (particularly 30-40% aerobic and 60-70% anoxic) but any alternation of anoxic-aerobic conditions may cause a problem in single reactor, two reactor, or multireactor systems in which nitrate and/or nitrite are present throughout the anoxic period, or in the anoxic reactor just prior to the aerobic reactor. Modern plants incorporating denitrification and/or phosphorus removal are obvious candidates for bulking and foaming due to *Microthrix*.

Figures 1 and 2 show typical views of *Microthrix* by using light microscopy and scanning electron microscopy respectively. It is not difficult to recognize using standard staining and microscopy, giving a positive response to Gram stain and being of fairly easily recognized morphology (Seviour *et al.* 1999). Of all the filaments creating difficulties in activated sludge plants, it is one of the most easily recognized, but there is a commercial test kit available which uses fluorescent situ hybridization (or "FISH") to permit visual identification should one feel the need.

The design of plants can play a significant part in the proliferation of scums and foams and there are many common mistakes in plant design which assist organisms like *Microthrix* by retaining floating masses in dead areas of the plant which have very high MCRT values and continuously reseed the biomass, (Pitman 1996). These should obviously be avoided (Figs 3, 5 and 6). Similarly poor mixing, poorly designed and inadequate aeration systems, cyclic overloading and low process D.O. levels can contribute to the creation of anoxic and anaerobic zones in what are supposed to be aeration basins.

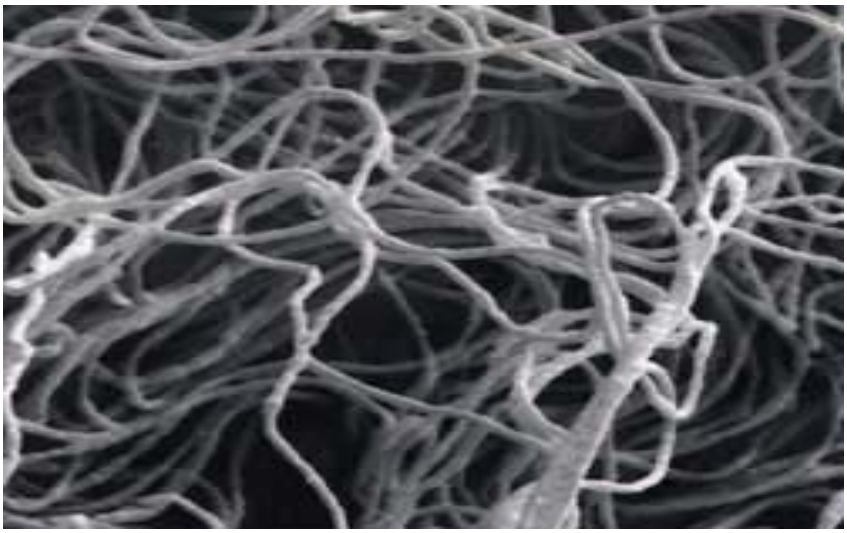


Figure 2. A scanning electron micrograph of Microthrix parvicella

Current Remedial Techniques

Jenkins *et al.* (1993) presented sludge chlorination as a method of choice in the United States to combat filamentous bulking due to any organism. The success of treatment of *Microthrix* in mixed liquor or foams is poor, due it is believed to resistant filamentous bacteria with hydrophobic cell walls such as *M. parvicella* and *Nostocoida limicola*.

Lakay *et al.* (1988) obtained only a partial elimination of *Microthrix parvicella* bacteria at a high chlorine dose. Hwang and Tanaka found in batch tests that *M. parvicella* remained intact at very high chlorine doses, while the microbial flocs were completely destroyed. Saayman *et al.* (1996) examined the use of non-specific chemical treatment in a BNR plant and assessed the effects of biomass settling characteristics and other operational parameters. While chlorine use was the most effective, it was reported to damage the biomass and cause difficulties in the P removal process when dosed at high levels, while ozone and peroxide were less effective in treating settling problems but less of a problem to the biomass.

Figure 3.
Dry Microthrix parvicella foam trapped in an anoxic zone of a BNR plant. aeration basin.

In recent times the introduction of selectors has been hailed as a major initiative in the control and elimination of filamentous bacteria (bulking and foaming) and the maintenance of moderate biomass SVIs. Evidence on the performance of selectors in controlling low F/M filaments has been described as both controversial and ambiguous and, in the Netherlands, despite incorporating over 80 selectors in full-scale plants, the percentage of plants with bulking associated with *Microthrix parvicella* was unchanged. Other experiences with the aerobic selector showed only little success in controlling the growth of *M. parvicella* in the presence of long chain fatty acids (LCFA), (Lebek and Rosenwinkel, 2002) and a comparison of anoxic selectors at five plants in the US has demonstrated that performance and effectiveness varied significantly (Marten and Daigger, 1997).

Figure 4. Typical dark brown Microthrix parvicella foam on an

More on Microthrix

Mamais *et al.* 1998 examined the effect of factors such as temperature, substrate type (easily biodegradable in the form of acetate and slowly biodegradable in the form of oleic acid) on Microthrix parvicella growth using complete mix with and without selectors (anoxic and anaerobic) and plug flow reactors. The results indicate that low temperatures and substrates in the form of long chain fatty acids favor the growth of *M. parvicella*. The plug flow configuration was shown to be quite effective in controlling the growth of *M. parvicella* and producing a sludge with good settling characteristics, while the presence of a selector, either anoxic or anaerobic, had no significant effect on the growth of *M. parvicella*. Maintenance of low sludge ages (5) days has also been reported to eliminate *M. parvicella* because it is a slow growing organism, but this is not always operationally possible.

While it is often convenient to group filaments together, it does appear the *Microthrix* has received special attention because of its ability to proliferate. More selective investigation of *Microthrix* has indicated that it has quite well defined requirements. The nature of *Microthrix* is such that it has the capability of using long chain fatty acids (oleic acid) and their esters (triglycerides of palmitic and stearic acid) (fats and oils) as sources of carbon and energy.

Lipids and LCFA are present in all domestic wastewater streams and often constitute a significant part of it. Values of 25-35% of the incoming COD have been reported, and it can support a substantial biomass production in a treatment plant. LCFA are generally easily consumed in activated sludge, and the consumption rate of LCFA under aerobic or anoxic conditions has been found to be rapid.

Studies indicate that *M.parvicella* consumes exclusively long chain fatty acids (LCFA), and that it is able to take up LCFA not only under aerobic, but also under anaerobic and anoxic conditions (Andreasen, K. and Nielsen, P.H. (2000)). It has been reported that *M. parvicella* is able to out-compete other bacteria particularly well in alternating anaerobic-aerobic and anoxic activated sludge systems. This ability is based on a high uptake and storage capacity for LCFA under anaerobic conditions and a subsequent use of the stored substrate for growth with oxygen (or nitrate) as electron acceptor.

Rosetti *et al.* (2002) carried out an extensive examination of *M. parvicella* and found that it was a very versatile organism which could store organic carbon under anaerobic conditions using stored polyphosphate for energy (like the organisms responsible for phosphorus removal). Once exposed to aerobic conditions it would recover rapidly and resume growing. *Microthrix* has a high storage capacity under all operating conditions (anaerobic, anoxic and anaerobic). It has a high "substrate affinity" or low Ks, which means it competes well at low substrate concentration.

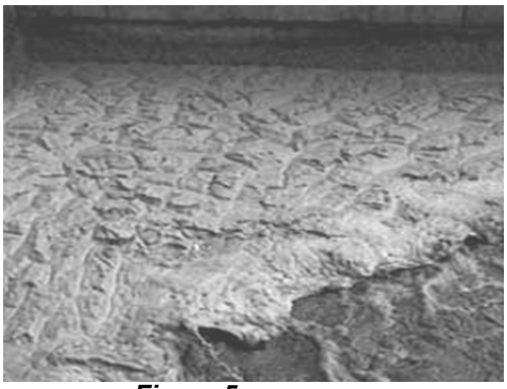


Figure 5.
Microthrix parvicella foam trapped near a mechanical aerator.

Most interestingly, *M. parvicella* has a maximum growth rate near 22° C, zero growth rate at 30° C and is capable of quite reasonably large growth rates at as low as 7° C which gives it a significant advantage in the competition with floc formers during winter in cold climates.

PAX vs. Microthrix parvicella

Microthrix parvicella is well-equipped to survive, compete and dominate in all kinds of activated sludge systems. With all of the above in mind, it is pleasing to find that Microthrix does have a weakness. That weakness is its apparent sensitivity to poly aluminum chloride (PAX) dosing, which seems to attack the ability of Microthrix parvicella to use lipids by reducing the activity of extracellular enzymes (lipases) on the surface of the organism rendering the organism relatively uncompetitive (Nielsen et al. 2003).

Roels *et al.* (2002) reported a loss of surface scum following PAX-14 dosing which was probably due to a loss of hydrophobicity. Full-scale dosages of PAX-14 range from 1.5 to 4.5 g Al^{3+}/kg MLSS/day depending on the sludge retention time (SRT); the lower the SRT, the higher the dosage and certainly lower than 7 g Al^{3+}/kg MLSS. Roels *et al.* (2002) offered the following empirical formula to establish the dose:

60/SRT = #g of Al^{3+}/kg MLSS

They also recommended the removal of the scum layer before dosing to allow the concentration and time of dosage to be kept at a minimum. Removal of the floating sludge layer from the surface before starting PAX application was necessary to ensure specific and rapid impact of Al-salts on *M. parvicella*.

In fact, the stable floating sludge represents an independent microbial system, into which aluminum can penetrate only at a limited extent. Dosage should be combined with high oxygen concentration in the aeration (i.e. above 2.5 mg/L) and the MLSS concentration low (i.e. under 2.5 g/L) since *M. parvicella* competes well at low oxygen levels.

Figure 6. A heavy build-up of trapped Microthrix parvicella foam during winter.

Of note was that the morphological properties of only Microthrix parvicella changed, apparently leaving the other filaments remaining unaffected.

Paris *et al.* (2003) came to a similar conclusion; by dosing $AlCl_3$ (3.5 mg mgAl^{3+} gMLSS/d), a general improvement of the settling properties of the activated sludge was achieved. As the filamentous population of activated sludge and the occurrence frequency of *M. parvicella* dropped, a decrease of hydrophobicity and floating tendency of activated sludge was observed. With low hydrophobicity the sludge does not tend to float. This has significant relevance for any measure to prevent floating foams.

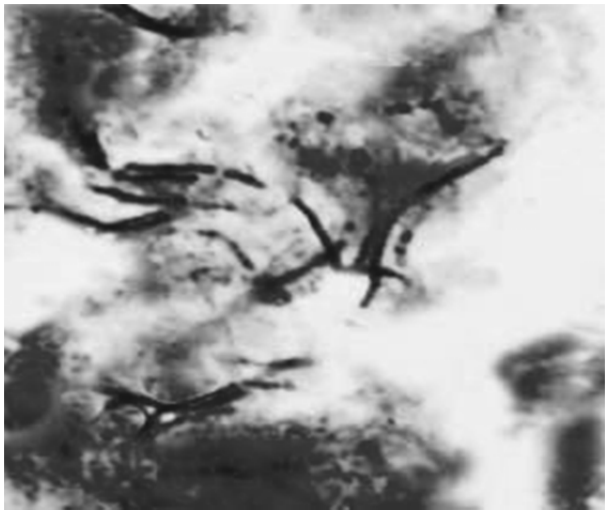


Figure 7. An typical view of Microthrix parvicella (gram stain x 1000) after extended PAX treatment.

It was observed that by adding PAX a morphological modification of the filamentous bacterium *M. parvicella* occurs. The morphological modification is probably the reason why the hydrophobic property of the filaments decreases. Paris *et al.* (2003) included micrographs which indicated that the *Microthrix parvicella* appeared to shorten in length after dosing (Figure 7) and no longer inhabit the zones between flocs.

PAX

PAX (or PAX-14 or polyaluminium chloride) used for *Microthrix* control is a flocculant or coagulant commonly used in water and wastewater treatment. The 14 or other number associated with the name refers to the particular grade of the chemical. Nielsen *et al.* (2003) report that PAX-14 is $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ and it is produced from $Al(OH)_3$ at high temperature and high pressure. PAX-14 and 18 are being used in several countries with good success for controlling *M. parvicella* - in particular Denmark where PAX-14 has been applied successfully in treatment plants with biological N and/or P removal for 91 out of 500 plants in 2002.

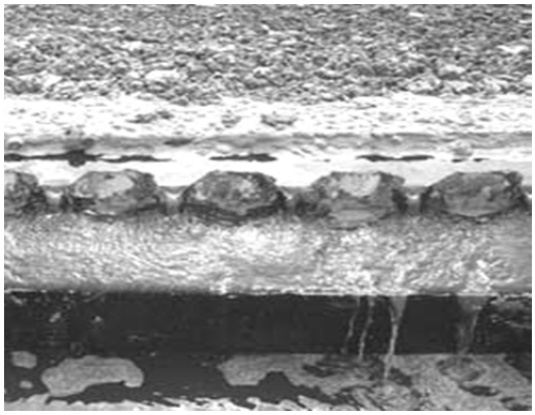


Figure 8. Foam build-up in a secondary clarifier resulting in solids loss and turbid effluent.

Proposed Treatment Regime

In the fall, to prevent the normal appearance of M.parvicella during the coming winter and to control problems with M. parvicella (winter, spring). **Dosage**: 0.5-1.5g Al/kgSS/day usually added to return sludge. PAX should be dosed continuously over the treatment period at the chosen level. Removal of floating sludge before and during dosing is recommended. Microscopic examination of the biomass and regular testing of biomass settling is also a very good idea and the dosing at the chosen remedial rate until a target SVI or preferably DSVI is reached should be the rule. It is not yet fully clear why PAX has the effect that it does, but the research continues. It is known that other Al salts have little effect on surface associated enzymes after 15 min, and no effect on surface hydrophobicity and surface associated enzymes.

Both photographs show the aeration sequence for an SBR.
Sequence Batch Reactor.

Anoxic zone, denitrification area.

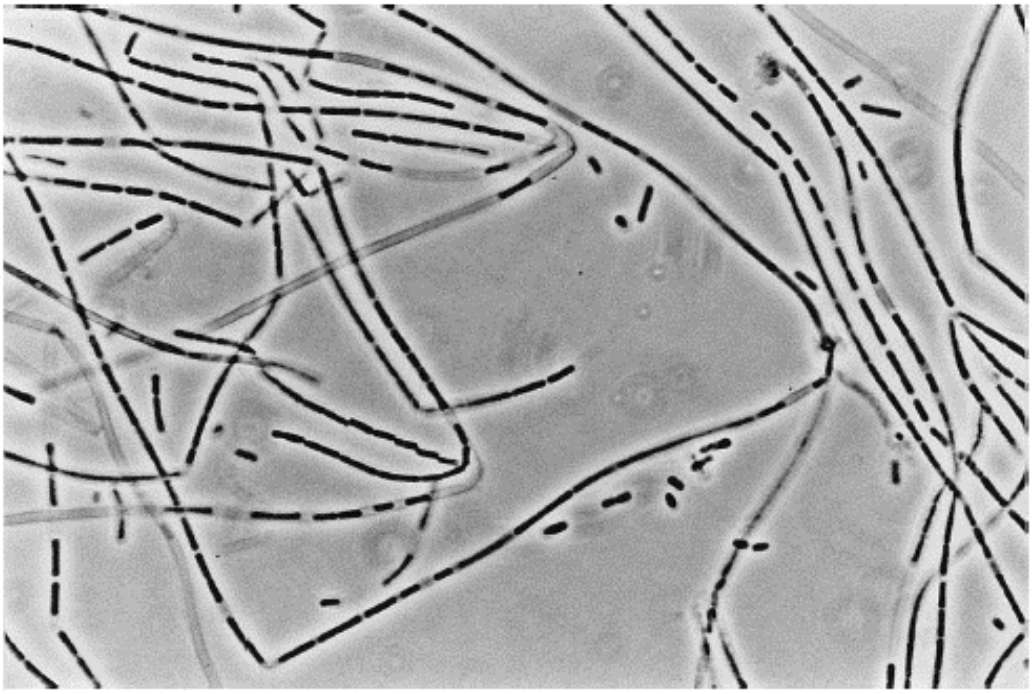
The same area from above photo, but clean and dry, these are porous air diffusers. A rare photo.

This clarifier is used to thicken sludge prior to the digester, this is not a digester but looks like one. Notice the light on the top for Operators to look inside.

Two massive anaerobic digesters.

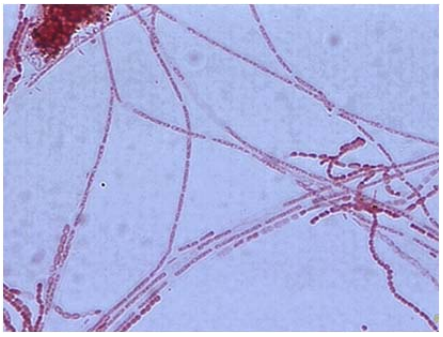
Sphaerotilus natans

Description and Significance
Sphaerotilus natans is a filamentous bacterium that is covered in a tubular sheath and can be found in flowing water and in sewage and wastewater treatment plants. While this bacterium sometimes clogs pipes and causes other similar problems, it does not cause major threat to wastewater treatment plants nor is it known to be pathogenic.



Long unbranched and ensheathed filaments produced by *Sphaerotilus natans* IF4.

Relatively long, non-motile filaments (100-1000 µm). Straight or smoothly curved with tree-like false branching. The cells are round-ended and rod shaped (1.0-1.8 x 1.5-3.0) and are contained in a clear, tightly fitting sheath. **Note:** They can be rectangular when the cells are tightly packed within the sheath. The cell septa are clear and easily observable with indentations. Filaments radiate outward from the floc surface into the bulk solution and can cause sludge settling interference by inter-floc bridging. The filament is usually Gram negative and Neisser negative. There are no sulfur granules. Poly-ß-hydroxybutric acid (PHB) is frequently observed as dark intracellular granules. In wastewater that is nutrient deficient, an exocellular slime coat may be present. Attached growth is usually uncommon, but may occur when at low growth rate.



This filament is usually found in environments where there is low DO or low nutrients (Nor P).

Control
RAS chlorination can be used to get rid of the filaments but process changes should also be made. Cell lysis occurs readily on this type of filament, although the empty sheaths still remain. Sludge wasting is necessary to remove them entirely from the system.

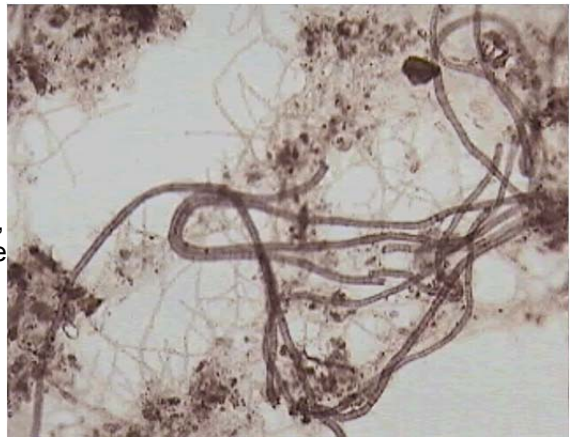
Manipulation of F/M and DO concentration can be used to control the filaments. Nutrient deficient wastes can be checked by effluent values of residual NH^3 and o-PO^4 and should be supplemented if necessary.

Rank
Sphaerotilus natans ranks 6th in number of predominance. Typically not found in pulp-mills with activated sludge.

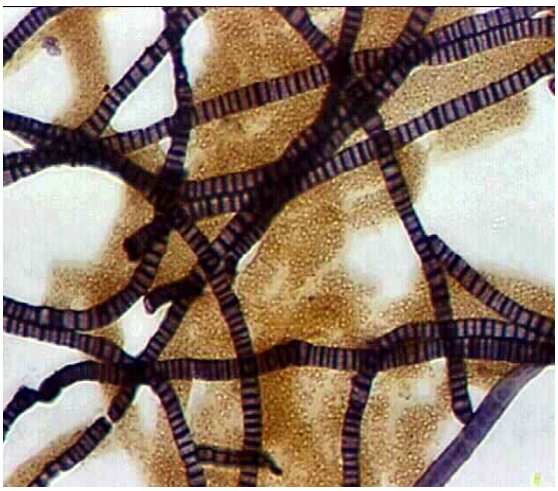
Nostocoida limicola *I* and *II*

Nostocoida limicola I is a bent and highly coiled filament. *N. limicola* has cells that are oval (0.6-0.8 µm wide) but are found to be closer to each other and the cell septa are almost indiscernible. The length of the filament can range from 100 to 200 µm and the majority of the time the trichome is found within the floc. *N. limicola* has no sheath and attached growth is rare. It stains Gram positive and Neisser positive.

***Nostocoida limicola II* Identification**
Medium length , non-motile filaments (100-200 µm). Bent and irregularly coiled filaments with incidental true branching. Knots sometimes seen. Cell septa are clear with indentations. Cells are oval or disc shaped (1.2-1.4 µm). Filaments are found within the floc structure but may occur in the bulk solution. The filament staining is variable, it is usually Gram negative but sometimes positive and Neisser positive. Usually easy to identify due to its Neisser staining properties. Stains entirely purple and looks like stacked discs (or hockey pucks). In industrial wastes, an organism that is Gram negative and Neisser negative occurs.



There is no sheath and there are no sulfur granules. Poly-ß-hydroxybutric acid (PHB) granules are frequently observed as dark intracellular granules. Attached growth is usually uncommon. Three subtypes are known. Resembles *M. parvicella* except in its Neisser staining properties.

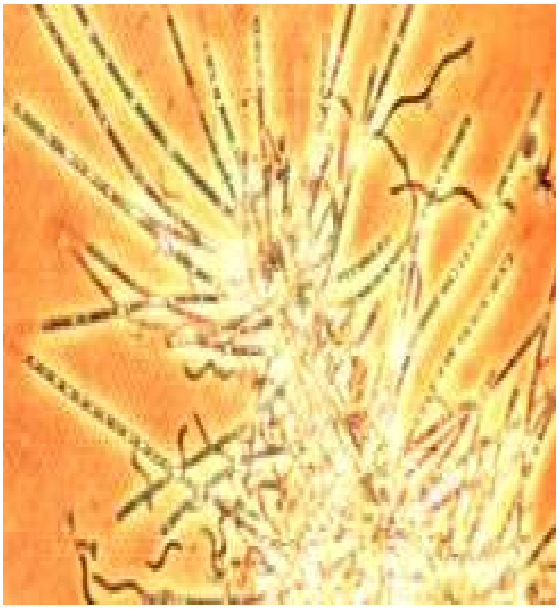


Environment
This filament is usually found in environments where there is low DO or low F/M and the presence of organic wastes. Wastes containing starch seem more selective to this filament. Bulking is more common in industrial wastes. The filament appears to be facultative fermentative, which is unique for most filaments.

Control
Manipulation of F/M (usually an increase) and DO concentration can be used to control the filaments. A selector may be used and chlorination. System changes include changing from a complete mix to plug flow aeration basin configuration.

N. limicola ranks 12th in number of predominance in industry. Typically not found in kraft mills. Common in municipalities.

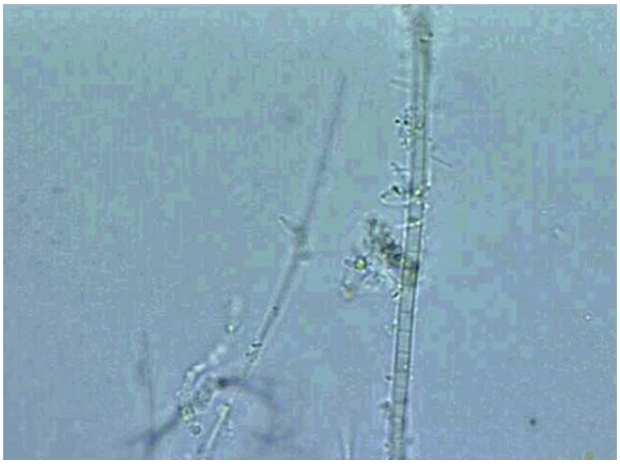
Thiothrix I & II



Thiothrix species consist of two types of Thiothrix and they are Thiothrix I and Thiothrix II. Thiothrix filaments are straight or slightly curved with Thiothrix I having an overall length of 100-500 µm and individual cells having a rectangular shape (1.4-2.5 x 3-5 µm). Thiothrix II has total length varying form 50-200 µm and its cells are rectangular (0.8-1.4 x 1-2 µm).

Both types of Thiothrix are found stretching from the floc surface, there is a noticeable septa between cells. Both species are Gram negative and Neisser negative with cells that on occasions have sulfur granules. There are additional structures on Thiothrix trichomes and they include apical gonidia as well as rosettes and a sheath is present, incidental attached growth may be observed. A holdfast may add to the characteristic of radiating out from a common center, the "starburst effect".

Relatively large, non-motile filaments (100-500 µm). Straight or smoothly curved filaments with no branching. Cells are rectangular (1.4 x 2.5 µm) and a clear cell septa is present without indentations at the septa. Filaments are found radiating outwards from the floc structure causing inter-floc bridging.

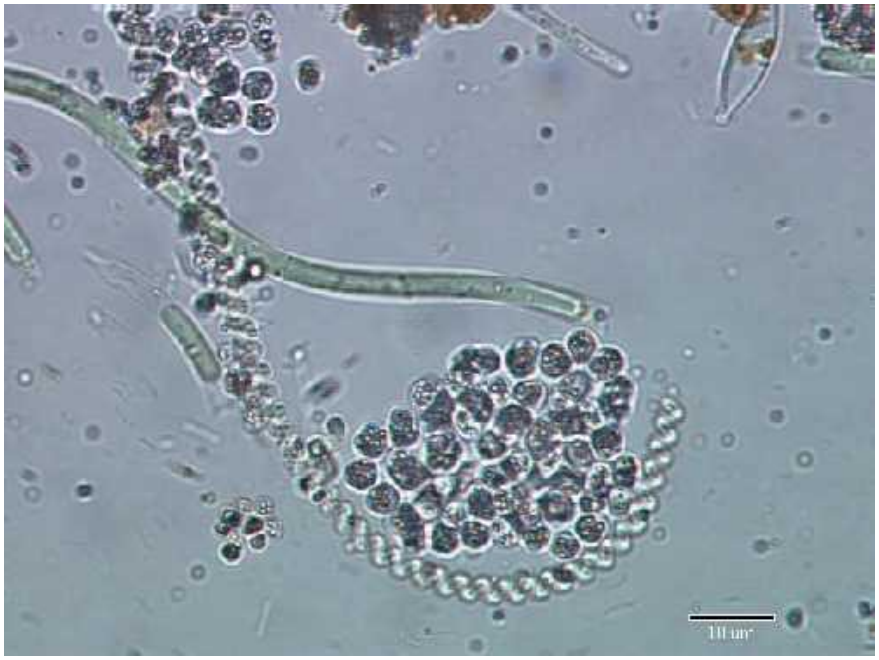


The filament staining is Gram negative or Gram variable when sulfur granules are present and Neisser negative with Neisser positive granules observed frequently.

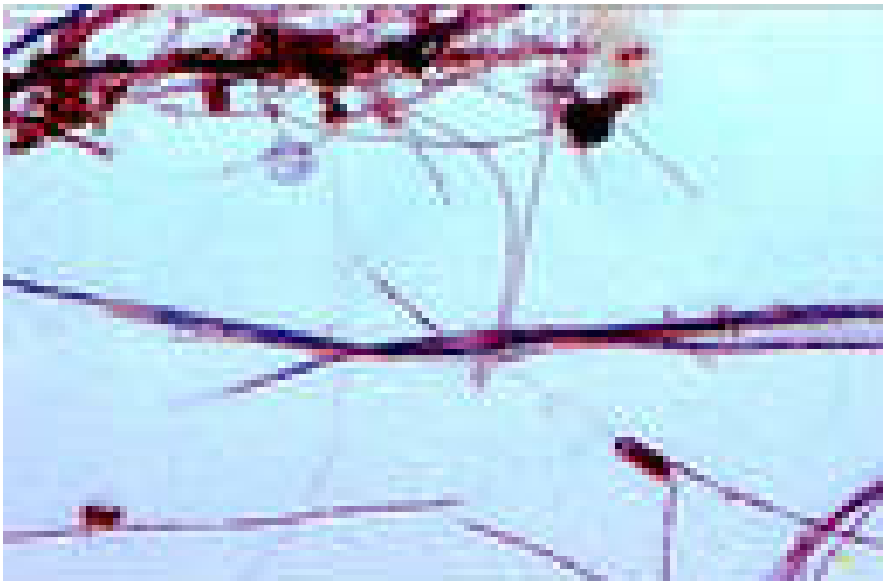
Exhibits bright sulfur granules in the presence of sulfides under phase contrast (use the S-test). Poly-ß-hydroxybutric acid (PHB) is frequently observed as dark intracellular granules. No attached growth when extending into the bulk solution. Can form rosettes and the filaments can have gonidia on the tips. Rosettes are when many filaments radiate outward from a common origin. Prominent heavy sheath. Easy to identify due to its large size.

Similar Organisms
Type 021N is similar when in the bulk solution and with no attached growth, although Type 021N has no sheath.

Environment
This filament is usually found in environments where there are limited nutrients (N or P). It can also be found in wastes containing specific compounds with sulfides and/or organic acids or environments with low DO. Sometimes found in plants with high pH in the aeration system.



Thiothrix II



Other Wastewater Treatment Components

Biochemical Oxygen Demand
Biochemical Oxygen Demand (**BOD or BOD5**) is an indirect measure of biodegradable organic compounds in water, and is determined by measuring the dissolved oxygen decrease in a controlled water sample over a five-day period.

During this five-day period, **aerobic** (oxygen-consuming) bacteria decompose organic matter in the sample and consume dissolved oxygen in proportion to the amount of organic material that is present. In general, a high BOD reflects high concentrations of substances that can be biologically degraded, thereby consuming oxygen and potentially resulting in low dissolved oxygen in the receiving water.

The BOD test was developed for samples dominated by oxygen-demanding pollutants like sewage. While its merit as a pollution parameter continues to be debated, BOD has the advantage of a long period of record.

Organic Carbon
Most organic carbon in water occurs as partly degraded plant and animal materials, some of which are resistant to microbial degradation. Organic carbon is important in the estuarine food web and is incorporated into the ecosystem by photosynthesis of green plants, then consumed as carbohydrates and other organic compounds by higher animals. In another process, formerly living tissue containing carbon is decomposed as detritus by bacteria and other microbes.

Total Organic Carbon
(**TOC**) bears a direct relationship with biological and chemical oxygen demand; high levels of TOC can result from human sources, the high oxygen demand being the main concern.

Microscopic identification is essential for any activated sludge process.

Lab tech removing filter for TSS analysis.

Here is an example of a rectangular clarifier used in the secondary settling process. Operation changes that should be employed if a dark brown foam is developing on the aeration basin is to increase the wasting rate.

Here is Pen floc being carried over the weir do to a process upset. Algae growth in excess can also create several different problems.

These operators are making sure that the backwash pumps are working for the sand filter. Notice the beautiful Arizona background.

During a plant upset, sludge from the filters can be carried over to the chlorine contact channel. In this photograph, it is not too bad, I've seen much worse.

Need for Nitrogen and Phosphorus Removal at Wastewater Treatment Plants

Nutrients

Nutrients are chemical elements or compounds essential for plant and animal growth. Nutrient parameters include ammonia, organic nitrogen, Kjeldahl nitrogen, nitrate nitrogen (for water only) and total phosphorus. High amounts of nutrients have been associated with eutrophication, or over-fertilization of a water body, while low levels of nutrients can reduce plant growth and (for example) starve higher level organisms that consume phytoplankton.

The purpose of this section is to provide an overview of the major factors driving decisions to enhance nutrient removal at WWTPs. This section characterizes the industry based on U.S. Environmental Protection Agency (EPA) survey information. This section describes the negative impacts of nutrient enrichment, highlighting the history of water quality changes in key regions of the country. EPA and State initiatives to reduce nutrient pollution from wastewater treatment discharges are summarized in this training course Lastly, we will highlight several barriers to enhancing nutrient removal at wastewater plants.

Status of Wastewater Treatment in the U.S.

The 1972 Amendments to the Federal Water Pollution Control Act (FWPCA)(Public Law 92-500), also known as the Clean Water Act (CWA), established the foundation for wastewater discharge control in the U.S. The CWA's primary objective is to "restore and maintain the chemical, physical, and biological integrity of the Nation's waters." The CWA established a program to ensure clean water by requiring permits that limit the amount of pollutants discharged by all municipal and industrial dischargers into receiving waters. Discharges are regulated under the National Pollutant Discharge Elimination System (NPDES) permit program. As of 2004, there were 16,583 municipal wastewater utilities [also known as Publicly Owned Treatment Works (POTWs)] regulated under the CWA, serving approximately 75 percent of the Nation's population (U.S. Public Health Service and USEPA, 2008) with the remaining population served by septic or other onsite systems.

Wastewater treatment has generally been defined as containing one or more of the following four processes: (1) preliminary, (2) primary, (3) secondary, and (4) advanced - also known as tertiary treatment.

Preliminary treatment consists of grit removal, which removes dense inert particles and screening to remove rags and other large debris. Primary treatment involves gravity settling tanks to remove settleable solids, including settleable organic solids. The performance of primary settling tanks can be enhanced by adding chemicals to capture and flocculate smaller solid particles for removal and to precipitate phosphorus. Secondary treatment follows primary treatment in most plants and employs biological processes to remove colloidal and soluble organic matter. Effluent disinfection is usually included in the definition of secondary treatment.

EPA classifies advanced treatment as "a level of treatment that is more stringent than secondary or produces a significant reduction in conventional, non-conventional, or toxic pollutants present in the wastewater" (U.S. Public Health Service and USEPA, 2008). Other technical references subdivide advanced treatment, using the terms "secondary with nutrient removal" when nitrogen, phosphorus, or both are removed and "tertiary removal" to refer to additional reduction in solids by filters or microfilters (Tchobanoglous et al, 2003). Effluent filtration and nutrient removal are the most common advanced treatment processes. The CWA requires that all municipal wastewater treatment plant discharges meet a minimum of secondary treatment. Based on data from the *2004 Clean Watersheds Needs Survey*, 16,543 municipal WWTPs (99.8 percent of plants in the country) meet the minimum secondary waste-water treatment requirements.

Of those that provide at least secondary treatment, approximately 44 percent provide some kind of advanced treatment (U.S. Public Health Service and USEPA, 2008).

Nutrient Impairment of U.S. Waterways

The harmful effects of eutrophication due to excessive nitrogen and phosphorus concentrations in the aquatic environment have been well documented. Algae and phytoplankton growth can be accelerated by higher concentrations of nutrients as they can obtain sufficient carbon for growth from carbon dioxide. In addition to stimulating eutrophication, nitrogen in the form of ammonia can exert a direct demand on dissolved oxygen (DO) and can be toxic to aquatic life. Even if a treatment plant converts ammonia to nitrate by a biological nitrification process, the resultant nitrate can stimulate algae and phytoplankton growth. Phosphorus also contributes to the growth of algae. Either nitrogen or phosphorus can be the limiting nutrient depending on the characteristics of the receiving water.

Nitrogen is typically limiting in estuarine and marine systems and phosphorus in fresh water systems. According to the 2007 report *Effects of Nutrient Enrichment in the Nation's Estuaries: A Decade of Change*, increased nutrient loadings promote a progression of symptoms beginning with excessive growth of phytoplankton and macroalgae to the point where grazers cannot control growth (Bricker et al., 2007). These blooms may be problematic, potentially lasting for months at a time and blocking sunlight to light-dependent submerged aquatic vegetation (SAV). In addition to increased growth, changes in naturally occurring ratios of nutrients may also affect which species dominate, potentially leading to nuisance/toxic algal blooms. These blooms may also lead to other more serious symptoms that affect biota, such as low DO and loss of SAV. Once water column nutrients have been depleted by phytoplankton and macroalgae and these blooms die, the bacteria decomposing the algae then consume oxygen, making it less available to surrounding aerobic aquatic life.

Consequently, fish and invertebrate kills may occur due to hypoxia and anoxia, conditions of low to no DO. Eutrophic conditions may also cause risks to human health, resulting from consumption of shellfish contaminated with algal toxins or direct exposure to waterborne toxins. Eutrophication can also create problems if the water is used as a source of drinking water. Chemicals used to disinfect drinking water will react with organic compounds in source water to form disinfection byproducts, which are potential carcinogens and are regulated by EPA.

Advanced eutrophic conditions can lead to "dead zones" with limited aquatic life, which describes the hypoxia condition that exists in the Northern Gulf of Mexico. A recent U.S. Geological Survey (USGS) report titled *Differences in Phosphorus and Nitrogen Delivery to the Gulf of Mexico from the Mississippi River Basin* documents the contribution of nitrogen and phosphorus from agricultural and non-agricultural sources in the Mississippi River basin (Alexander et al., 2008).

On June 16, 2008 the joint federal-state Mississippi River/Gulf of Mexico Watershed Nutrient Task Force released its *2008 Action Plan for Reducing, Mitigating, and Controlling Hypoxia in the Northern Gulf of Mexico and Improving Water Quality in the Mississippi River Basin,* which builds upon its 2001 plan by incorporating emerging issues, innovative approaches, and the latest science, including findings from EPA's Science Advisory Board.

Improvements include more accountability through an Annual Operating Plan, better tracking of progress, state and federal nutrient reduction strategies, and a plan to increase awareness of the problem and implementation of solutions (USEPA, 2008b). Nutrient pollution has also caused significant problems in the Chesapeake Bay. Elevated levels of both nitrogen and phosphorus are the main cause of poor water quality and loss of aquatic habitats in the Bay. Significant algae blooms on the water surface block the sun's rays from reaching underwater bay grasses. Without sunlight, bay grasses cannot grow and provide critical food and habitat for blue crabs, waterfowl, and juvenile fish.

The Chesapeake Bay Program estimates that 22 percent of the phosphorus loading and 19 percent of the nitrogen loading in the Bay comes from municipal and industrial wastewater facilities (Chesapeake Bay Program, 2008). The first national attention to nutrient contamination occurred in the Great Lakes. In the 1960s Lake Erie was declared "dead" when excessive nutrients in the Lake fostered excessive algae blooms that covered beaches and killed off native aquatic species due to oxygen depletion. At that time, phosphorus was the primary nutrient of concern due to the advent of phosphate detergents and inorganic fertilizers. With the enactment of the CWA and the Great Lakes Water Quality Agreement in 1972, a concerted effort was undertaken to reduce pollutant loadings, including phosphorus in the Lake.

Although the health of the Lake improved dramatically, in recent years, there has been renewed attention to the re-emergence of a "dead" zone in Lake Erie, again due to nutrient loadings. Recent studies by scientists and the National Oceanic and Atmospheric Administration (NOAA) have also hypothesized a relationship between excessive nutrients in the Lake and the presence of two aquatic invasive species – the zebra mussel and the quagga mussel (Vanderploeg et al., 2008). Development and population increases in the Long Island Sound Watershed have resulted in a significant increase in nitrogen loading to the Sound. The increased nitrogen loads have stimulated plant growth, increased the amount of organic matter settling to the benthic zone, lowered DO levels, and changed habitats.

The primary concerns in the Sound include hypoxia, the loss of sea grass, and alterations in the food web. Management efforts are currently underway to reduce nitrogen pollution by more than half with a focus on upgrading WWTPs with new technologies and removing nitrogen by reducing polluted run-off through best management practices on farms and suburban areas (Long Island Sound Study, 2004). The above represent four examples of impaired large water bodies impacted by nutrient loadings. There are more than 80 additional estuaries and bays, and thousands of rivers, streams, and lakes that are also impacted by nutrients in the U.S. In fact, all but one state and two territories have CWA section 303(d) listed1 water body impairments for nutrient pollution. Collectively, states have listed over 10,000 nutrient and nutrient–related impairments.

Climate change may also be a significant influence on the development of future eutrophic symptoms. According to the report *Effects of Nutrient Enrichment in the Nation's Estuaries: A Decade of Change*, the factors associated with climate change that are expected to have the greatest impacts on coastal eutrophication are:

- Increased temperatures
- Sea level rise
- Changes in precipitation and freshwater runoff

Increased temperatures will have several effects on coastal eutrophication. Most coastal species are adapted to a specific range of temperatures. Increases in water temperatures may lead to expanded ranges of undesirable species. Higher temperatures may also lead to increased algal growth and longer growing seasons, potentially increasing problems associated with excessive algal growth and nuisance/toxic blooms. Additionally, warmer waters hold less DO, therefore potentially exacerbating hypoxia. Temperature-related stratification of the water column may also worsen, having a further negative effect on DO levels.

Climate change models predict increased melting of polar icecaps and changes in precipitation patterns, leading to sea level rise and changes in water balance and circulation patterns in coastal systems. Sea level rise will gradually inundate coastal lands, causing increased erosion and sediment delivery to water bodies, and potentially flooding wetlands. The increased sediment load and subsequent turbidity increase may cause SAV loss. The positive feedback between increased erosion and algal growth (as erosion increases, sediment associated nutrients also increase, stimulating growth) may also increase turbidity. The loss of wetlands, which act as nutrient sinks, will further increase nutrient delivery to estuaries.

Another report titled *Aquatic Ecosystems and Global Climate Change – Potential Impacts on Inland Freshwater and Coastal Wetland Ecosystems in the United States* notes that climate change of the magnitude projected for the U.S. over the next 100 years will cause significant changes to temperature regimes and precipitation patterns across the U.S. (Poff et al., 2002). Such alterations in climate pose serious risks for inland freshwater ecosystems (lakes, streams, rivers, wetlands) and coastal wetlands, and may adversely affect numerous critical services provided to human populations.

These conclusions indicate climate change is a significant threat to the species composition and function of aquatic ecosystems in the U.S. However, critical uncertainties exist regarding the manner in which specific species and whole ecosystems will respond to climate change. These arise both from uncertainties about how regional climate will change and how complex ecological systems will respond.

Indeed, as climate change alters ecosystem productivity and species composition, many unforeseen ecological changes are expected that may threaten the goods and services that these systems provide to humans. Required by Section 303(d) of the CWA, the 303(d) list is a list of state's water bodies that do not meet or are not expected to meet applicable Water Quality Standards with technology-based controls alone.

Federal and State Initiatives to Reduce Nutrient Pollution
NPDES Permitting
Established by the FWPCA Amendment of 1972, EPA's NPDES permit program has been the primary mechanism for controlling pollution from point sources. Point sources are discrete conveyances such as pipes or man-made ditches. Individual homes that are connected to a municipal system, use a septic system, or do not have a surface discharge do not need an NPDES permit; however, POTWs and other facilities must obtain permits if they discharge directly to surface waters.

NPDES permits for wastewater discharges contain, among other information, effluent limits for "conventional" pollutants such as biochemical oxygen demand (BOD), total suspended solids (TSS), and pH as well as limits for specific toxicants including various organic and inorganic chemicals. Permits may also include effluent limits for "non-conventional" pollutants such as nitrogen and phosphorus. Effluent limits can be technology-based and/or water-quality based. EPA has established technology-based, secondary treatment effluent limits for BOD as 5-day biochemical oxygen demand (BOD5), TSS, and pH.

Water-quality based effluent limits are set if the technology-based limits are not sufficient to maintain the water quality standards (WQS) of the receiving water. Federal and State regulations related to WQSs and Total Maximum Daily Loads (TMDLs) are expected to drive down NPDES effluent limits for nitrogen and phosphorus. WQS define the goals for a water body by designating its uses, setting criteria to protect those uses, and establishing provisions to protect water bodies from pollutants. Criteria can be narrative or numeric.

Regulatory agencies can adopt *nutrient criteria* to protect a water body against nutrient over-enrichment and eutrophication caused by nitrogen and phosphorus. In June 1998, EPA issued a *National Strategy for the Development of Regional Nutrient Criteria.* This was followed by publication of recommended nutrient criteria for most streams and lakes in 2001. In a January 9, 2001 *Federal Register* notice, EPA recommended that states and other regulatory agencies develop a nutrient criteria plan to outline their process for adopting such nutrient criteria (*Federal Register*, 2001).

As of May 2007, only a handful of States and Territories had adopted nutrient criteria for nitrogen and phosphorus (USEPA, 2007a), although many have made progress in criteria development.

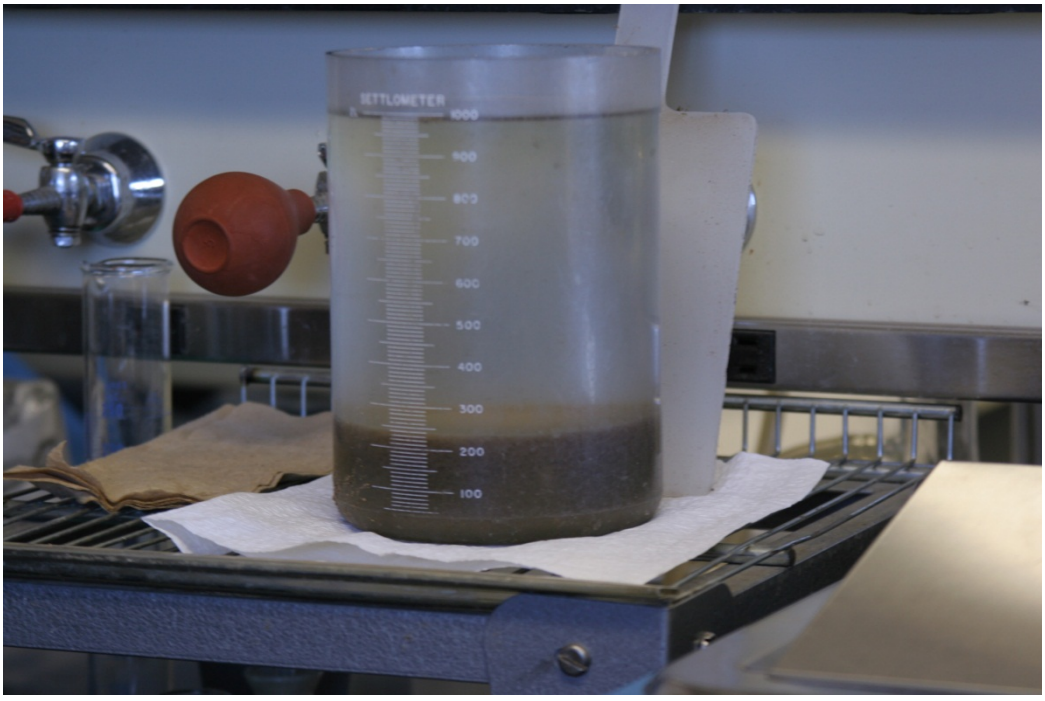
In a memo dated May 25, 2007, EPA encouraged all regulatory agencies to "…accelerate their efforts and give priority to adopting numeric nutrient standards or numeric translators for narrative standards for all waters in States and Territories that contribute nutrient loadings to our waterways" (USEPA, 2007b).

CWA Section 303(d) requires states to develop TMDLs for water bodies on the 303(d) list of impaired waters. A TMDL is a calculation of the maximum amount of a pollutant a water body can receive and still meet WQS. TMDLs serve as a tool for implementing WQS. The TMDL targets or endpoints represent a number where the applicable WQS and designated uses (e.g., such as public water supply, contact recreation, and the propagation and growth of aquatic life) are achieved and maintained in the water body of concern.

TMDLs identify the level of pollutant control necessary to meet WQS and support the designated uses of a water body. Once a TMDL is set, the total load is allocated among all existing sources. The allocation is divided into two portions - a load allocation representing natural and non-point sources and a waste load allocation representing NPDES permitted point source discharges. In many regions, water bodies have a poor ability to assimilate nutrients or water bodies are already impaired from past pollution and the water body cannot handle large loads of additional nutrients. In these cases, TMDLs may require nutrient permit levels to be even lower than what might be allowed otherwise by nutrient criteria.

No need for operators at the WWT facility, this oxidation ditch is overseen by ducks. Lucky for them the operators do a great job. It is very common to have all types of waterfowl and birds at your facility. I've seen Eagles to Cranes, EVEN BEARS.

The spinning reel for this oxidation ditch is mixing or aerating properly.



This is a 1000 ml settlometer used to determine the Sludge Volume Index (SVI). Increase sludge wasting to decrease MCRT; this may prevent sludge from floating to the surface of a secondary clarifier. Sludge that is rising to the top of the clarifier is a good indication that sludge is not being removed from the primary clarifier often enough.

Water Quality Trading

Water quality trading is a market-based approach to improve and preserve water quality. Trading can provide greater efficiency in achieving water quality goals by allowing one source to meet its regulatory obligations by using pollutant reductions created by another source that has lower pollution control costs. For example, under a water quality trading program, a POTW could comply with discharge requirements by paying distributed sources to reduce their discharges by a certain amount. The use of geographically-based trading ratios provides an economic incentive, encouraging action toward the most cost effective and environmentally beneficial projects.

EPA issued a Water Quality Trading Policy in 2003 to provide guidance to States and Tribes on how trading can occur under the CWA and its implementing regulations. The policy discusses CWA requirements that are relevant to water quality trading including: requirements to obtain permits, antibacksliding provisions, development of WQSs including an antidegradation policy, NPDES permit regulations, TMDLs and water quality management plans. EPA also developed a number of tools and guidance documents to assist states, permitted facilities, non-point sources, and stakeholders involved in the development of trading programs (www.epa.gov/owow/watershed/trading.htm). Recently, the U.S. Department of Agriculture (USDA) National Resources Conservation Service released a Nitrogen Trading Tool (NTT) prototype for calculating nitrogen credits based on the Nitrogen Loss and Environmental Assessment Package Model (Gross et al., 2008).

Water quality trading programs have been successfully implemented in several states and individual watersheds across the county. For example, nitrogen pollution from point sources into the Long Island Sound was reduced by nearly 25 percent using an innovative Nitrogen Credit Trading Program. In Connecticut, the program was implemented among 79 sewage treatment plants in the state. Through the Nitrogen Credit Exchange, established in 2002, the Connecticut program has a goal of reducing nitrogen discharges by 58.5 percent by 2014. A recent American Society of Civil Engineers journal article points out, however, that regulatory frameworks for water quality trading programs have yet to be adopted by the majority of States. Barriers to adopting such programs include uncertainty in: (1) the mechanisms for determining appropriate credits and ratios between point sources and distributed sources; and (2) approaches to ensure that promised reductions actually occur (Landers, 2008).

Aeration is often used to refresh the wastewater flow at the influent channel.

The photograph above and below are of an operator taking mixed liquor samples in an oxidation ditch. Always wear latex gloves, many operators quit wearing gloves after a short period of time. Don't become unafraid of the pathogens. Fear the bugs no matter what.

Nutrient Constituents in Wastewater and Measurement Methods

This section provides an overview of the sources, forms, and measurement methods for nitrogen and phosphorus in wastewater.

Nitrogen
Nitrogen is an essential nutrient for plants and animals. Approximately 80 percent of the earth's atmosphere is composed of nitrogen and it is a key element of proteins and cells. The major contributors of nitrogen to wastewater are human activities such as food preparation, showering, and waste excretion. The per capita contribution of nitrogen in domestic wastewater is about 1/5th of that for BOD. Total nitrogen in domestic wastewater typically ranges from 20 to 70 mg/L for low to high strength wastewater (Tchobanoglous et al., 2003). Factors affecting concentration include the extent of infiltration and the presence of industries. Influent concentration varies during the day and can vary significantly during rainfall events, as a result of inflow and infiltration to the collection system.

The most common forms of nitrogen in wastewater are:
• Ammonia (NH_3)
• Ammonium ion (NH_4+)
• Nitrite (NO_2-)
• Nitrate (NO_3-)
• Organic nitrogen

Nitrogen in domestic wastewater consists of approximately 60 to 70 percent ammonia-nitrogen and 30 to 40 percent organic nitrogen (Tchobanoglous et al., 2003; Crites and Tchobanoglous, 1998). Most of the ammonia-nitrogen is derived from urea, which breaks down rapidly to ammonia in wastewater influent. EPA approved methods for measuring ammonia, nitrate, and nitrite concentration use colorimetric techniques. Organic nitrogen is approximated using the standard method for Total Kjeldahl Nitrogen (TKN) (APHA, AWWA, and WEF, 1998).

The TKN method has three major steps:
(1) digestion to convert organic nitrogen to ammonium sulfate;
(2) conversion of ammonium sulfate into condensed ammonia gas through addition of a strong base and boiling; and
(3) measurement using colorimetric or titration methods. Because the measured concentration includes ammonia, the ammonia-nitrogen concentration is subtracted from the TKN to determine organic nitrogen.

Nitrogen components in wastewater are typically reported on an "as nitrogen" basis so that the total nitrogen concentration can be accounted for as the influent nitrogen components are converted to other nitrogen compounds in wastewater treatment.

WWTPs designed for nitrification and denitrification can remove 80 to 95 percent of inorganic nitrogen, but the removal of organic nitrogen is typically much less efficient (Pehlivanoglu-Mantas and Sedlak, 2006). Domestic wastewater organic nitrogen may be present in particulate, colloidal or dissolved forms and consist of proteins, amino acids, aliphatic N compounds, refractory natural compounds in drinking water (e.g. Humic substances), or synthetic compounds (e.g. ethylene Diamine tetraacetic acid (EDTA)). Organic nitrogen may be released in secondary treatment by microorganisms either through metabolism or upon death and lysis. Some nitrogen may be contained in recondensation products. Hydrolysis of particulate and colloidal material by microorganisms releases some organic nitrogen as dissolved, biodegradable compounds. Amino acids are readily degraded during secondary biological treatment, with 90 to 98 percent removal in activated sludge systems and 76 to 96 percent removal in trickling filters. However, other forms of organic nitrogen may be more persistent in wastewater treatment processes.

The importance of organic nitrogen has increased as effluent limits on nitrogen have become more stringent. With more impaired waterways from nutrient loads, effluent limits for total nitrogen (TN) concentrations of 3.0 mg/L or less are becoming more common. The dissolved organic nitrogen (DON) concentration in the effluent from biological nutrient removal treatment facilities was found to range from 0.50 to 1.50 mg/L in 80 percent of 188 plants reported by Pagilla (STAC-WERF, 2007) and values as high as 2.5 mg/L were observed. Thus, for systems without effluent filtration or membrane bioreactors (MBRs) that are trying to meet a TN treatment goal of 3.0 mg/L, the effluent DON contribution can easily be 20 to 50 percent of the total effluent nitrogen concentration, compared to only about 10 percent for conventional treatment (Pehlivanoglu-Mantas and Sedlak, 2004).

The chemical composition of DON in wastewater effluents is not completely understood. Sedlak (2007) has suggested that only about 20 percent of the DON has been identified as free and combined amino acids, EDTA, and other trace nitrogen compounds. About 45 percent may be unidentified low molecular weight compounds and the other 35 percent as unidentified high molecular weight compounds containing Humic acids and amides. Similar results were found by Khan (2007). Early work by Parkin and McCarty (1981) suggested that 40 to 60 percent of effluent DON is non-bioavailable. The non-bioavailable portion is also referred to as recalcitrant DON (rDON).

Primary clarifier

Phosphorus

Total phosphorus (TP) in domestic wastewater typically ranges between 4 and 8 mg/L but can be higher depending on industrial sources, water conservation, or whether a detergent ban is in place. Sources of phosphorus are varied. Some phosphorus is present in all biological material, as it is an essential nutrient and part of a cell's energy cycle. Phosphorus is used in fertilizers, detergents, and cleaning agents and is present in human and animal waste.

Phosphorus in wastewater is in one of three forms:
• Phosphate (also called Orthophosphate)
• Polyphosphate, or
• Organically bound phosphorus.

The orthophosphate fraction is soluble and can be in one of several forms (e.g., phosphoric acid, phosphate ion) depending on the solution pH. Polyphosphates are high-energy, condensed phosphates such as pyrophosphate and trimetaphosphate. They are also soluble but will not be precipitated out of wastewater by metal salts or lime. They can be converted to phosphate through hydrolysis, which is very slow, or by biological activity.

Organically bound phosphorus can either be in the form of soluble colloids or particulate. It can also be divided into biodegradable and non-biodegradable fractions. Particulate organically bound phosphorus is generally precipitated out and removed with the sludge. Soluble organically bound biodegradable phosphorus can be hydrolyzed into orthophosphate during the treatment process.

Soluble organically bound non-biodegradable phosphorus will pass through a wastewater treatment plant. A typical wastewater contains 3 to 4 mg/L phosphorus as phosphate, 2 to 3 mg/L as polyphosphate, and 1 mg/L as organically bound phosphorus (WEF and ASCE, 2006).

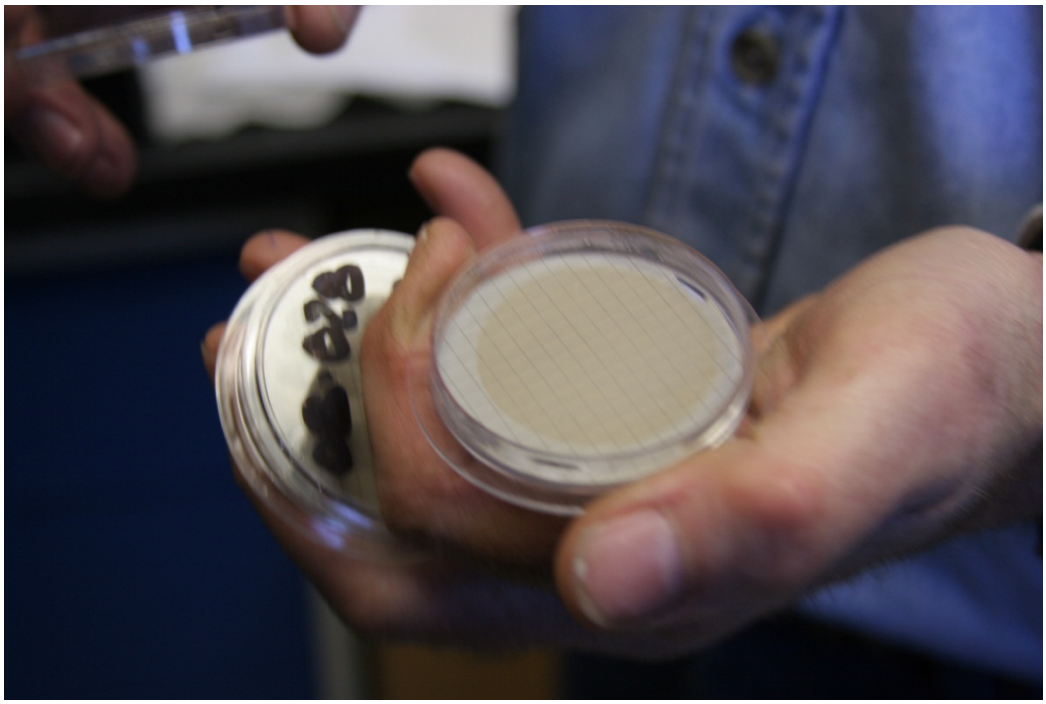
Phosphorus content in wastewater can be measured as
• Orthophosphate
• Dissolved orthophosphate
• Total phosphorus
• Total dissolved phosphorus (i.e., all forms except particulate organic phosphorus)

EPA approved laboratory methods rely on colorimetric analysis. Colorimetric analysis measures orthophosphate only, so a digestion step is needed to convert polyphosphate and organic phosphorus to orthophosphate to measure TP. The persulfate method is reported to be the most common and easiest method (WEF and ASCE, 2006). To determine dissolved phosphorus (either total dissolved phosphorus or total dissolved orthophosphate), the sample is first filtered through a 0.45 micron filter.

USEPA approved colorimetric methods are routinely used to measure phosphorus levels as low as 0.01 mg/L. On-line analyzers that use the colorimetric method are available from venders (e.g., the Hach PhosphaxTM SC phosphate analyzer).

Ion chromatography is a second common technique used to measure orthophosphate in wastewater. As with colorimetric methods, digestion is required for TP measurement, with persulfate digestion recommended (WEF and ASCE, 2006).

Microscopes are used to see indicator bugs and other MO's microorganisms. This examination is used so that the operator knows how well the process is working.



This is a filter used for the coliform test.

Phosphorus Removal by Chemical Addition

The purpose of this section is to describe techniques for phosphorus removal by chemical addition. It summarizes issues associated with chemical feed location, mixing, and sludge production. An overview of advanced solids separation processes is also provided.

Principles

Chemical precipitation for phosphorus removal is a reliable, time-tested, wastewater treatment method that has not drastically changed over the years. To achieve removal, various coagulant aids are added to wastewater where they react with soluble phosphates to form precipitates. The precipitates are removed using a solids separation process, most commonly settling (clarification). Chemical precipitation is typically accomplished using either lime or a metal salt such as aluminum sulfate (alum) or ferric chloride. The addition of polymers and other substances can further enhance floc formation and solids settling. Operators can use existing secondary clarifiers or retrofit primary clarifiers for their specific purposes.

Aluminum and Iron Salts

Alum and ferric or ferrous salts are commonly used as coagulant and settling aids in both the water and wastewater industry. They are less corrosive, create less sludge, and are more popular with operators compared to lime. Alum is available in liquid or dry form, can be stored on site in steel or mild concrete, and has a near unlimited shelf life. Ferric chloride is similar although care is needed during handling because of corrosivity. If an industrial source is available such as waste pickle liquor, ferrous chloride or ferrous sulfate have been used for phosphorus removal. Ferrous forms should be added directly to aerobic reactors rather than to anaerobic reactors such as primary settling basins because the ferrous iron needs to oxidize to ferric iron for best results.

The molar ratio of aluminum to phosphorus required for phosphorus removal ranges from about 1.38:1 for 75 percent removal, 1.72:1 for 85 percent removal, and 2.3:1 for 95 percent removal.

For iron compounds, a ratio of about 1:1 is required, with a supplemental amount of iron (10 mg/L) added to satisfy the formation of hydroxide (WEF and ASCE, 1998). For additional removal of phosphorus with aluminum and iron salts, a ratio of between 2 and 6 parts metal salt to 1 part phosphorus may be required for adequate phosphorus removal.

To supplement stoichiometry calculations, designers should consider jar tests and, in some cases, full-scale pilot tests to gauge the effects on the required dose of competing reactions; the influence of pH and alkalinity, adsorption, and co-precipitation reactions; and the interaction with polymers that are added to increase coagulation and flocculation (WEF and ASCE, 1998; Bott et al. 2007).

Aluminum or ferric iron salts can be added to the primary clarifier, secondary clarifier, tertiary clarifier, or directly into the activated sludge aeration tank. Multiple additions can increase phosphorus removal efficiency. Ferrous salts can only be added to the aeration basin since it needs to be oxidized to ferric to precipitate the phosphorus.

The solubility of aluminum and iron salts is a function of pH. The optimum solubility for alum was previously reported to occur at a pH range of 5.5 to 6.5, significantly lower than most influent wastewater. Recent studies (Szabo et al., 2008) showed that the range for both iron and alum is between 3.5 and 7.5 with the highest efficiency between pH 5.5 and 7.

Chemicals such as lime compounds, caustic soda, and soda ash can be used to raise the pH of the waste stream prior to biological treatment processes or discharge. It is important to understand that alkalinity is consumed during the precipitation reactions, and precipitation will be incomplete if insufficient alkalinity is present.

Lime

Although lime had lost favor due to issues associated with chemical handling, sludge production, and re-carbonation, it has recently been considered more often because of its ability to reduce phosphorus to very low levels when combined with effluent filtration and the microbial control properties associated with its high pH. When lime is added to wastewater, it first reacts with the bicarbonate alkalinity to form calcium carbonate (CaCO3). As the pH increases to more than 10, excess calcium ions will react with phosphate to precipitate hydroxylapatite [CA5(OH)(PO4)3].

Because it reacts first with alkalinity, the lime dose is essentially independent of the influent phosphorus concentration. Tchobanoglous et al. (2003) estimates the lime dose to typically be 1.4 to 1.6 times the total alkalinity expressed as CaCO3.

The typical reaction between calcium compounds and phosphorus is represented below:
5Ca2+ + 4OH- + 3HPO4- □ Ca5OH(PO4)3 + 3H2O (4-3)

The molar ratio required for phosphorus precipitation with lime is approximately 5:3, but can vary from between 1.3 to 2, depending on the composition of the wastewater. As with iron and aluminum salts, jar tests can be used to determine correct doses for a specific wastewater stream (WEF,1998).

Lime addition can raise the pH to greater than 11. Because activated sludge processes require pH levels below 9, lime cannot be added directly to biological treatment processes or it will cause process upsets. Lime can be added to primary sedimentation tanks and removed with the primary sludge or it can be added as a tertiary treatment process after biological treatment. When added to primary tanks, it will also result in the removal of colloidal material through coagulation and settling, with a concomitant removal of TSS up to 80 percent and chemical oxygen demand (COD) up to 60 percent.

In either case, pH adjustment is needed and typically accomplished by adding CO2 or a liquid acid such as sulfuric acid, nitric acid, or hypochlorite (Tchobanoglous et al., 2003; USEPA, 1999a).

Hortskotte et al. (1974) showed that when the primary effluent is discharged directly to a nitrifying activated sludge plant, the hydrogen ions produced may neutralize the high pH. However, when denitrification is practiced and the operator wishes to make use of the soluble COD in the primary effluent, the effluent must be neutralized before discharging it to the anoxic zone.

Lime requires special handling and operations practices that further set it apart from chemical precipitation by metal salts. Although the formation of carbonate scaling on equipment and pipes is a drawback of lime treatment, lime slaking, where quicklime (CaO) is reacted with water to form calcium hydroxide (Ca(OH)2), is the biggest operational disadvantage.

Location of Chemical Feed and Mixing

Lime or metal salts can be added at several locations throughout the treatment plant to remove phosphorus.

“Pre-precipitation” is when chemicals are added to raw water to precipitate phosphorus in the primary sedimentation basins.

“Co-precipitation” involves adding chemicals to form precipitates that can be removed with biological sludge.

“Post-precipitation” is when chemicals are added after secondary sedimentation and precipitants are removed in a tertiary process such as sedimentation or filtration (Tchobanoglous et al., 2003). Because it requires a high pH to achieve a low phosphorus concentration, lime cannot be added directly to biological reactors or to the secondary clarifiers.

Multipoint additions of iron or aluminum salts have been very effective and can typically remove more phosphorus than single-point applications. There are several advantages to post-precipitating phosphorous using a tertiary treatment technique (after biological processes in a separate reactor):

• Microorganisms rely on phosphorus as a food source. If too much phosphorus is removed prior to biological treatment, biological processes may suffer. For activated sludge, the minimum ratio of phosphorus to BOD5 for a rapidly growing (low solids retention time (SRT)) system is typically about 1:100 (WEF and ASCE, 1998).

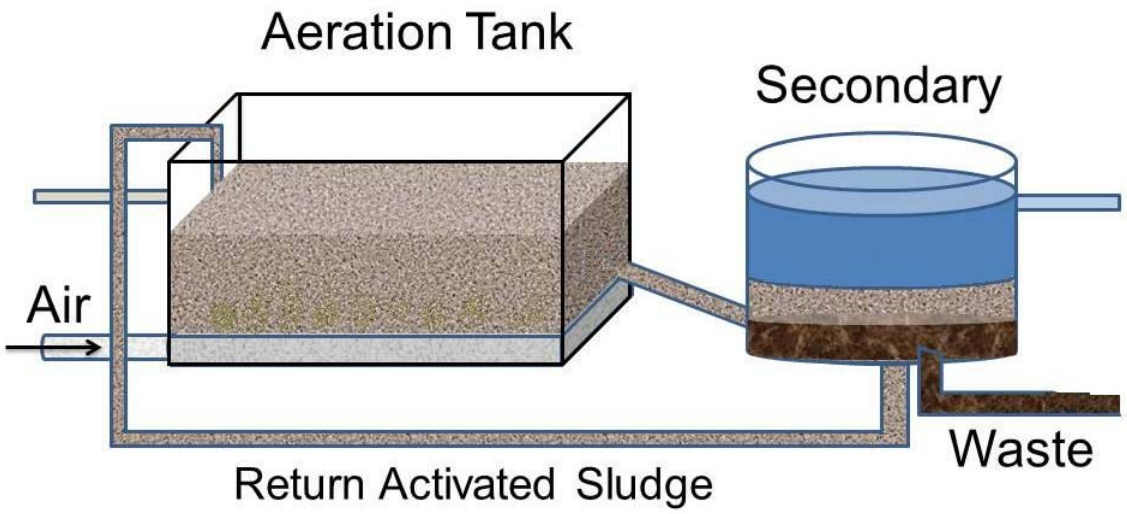
• Competing chemicals in the primary sedimentation basins can increase the required dose.

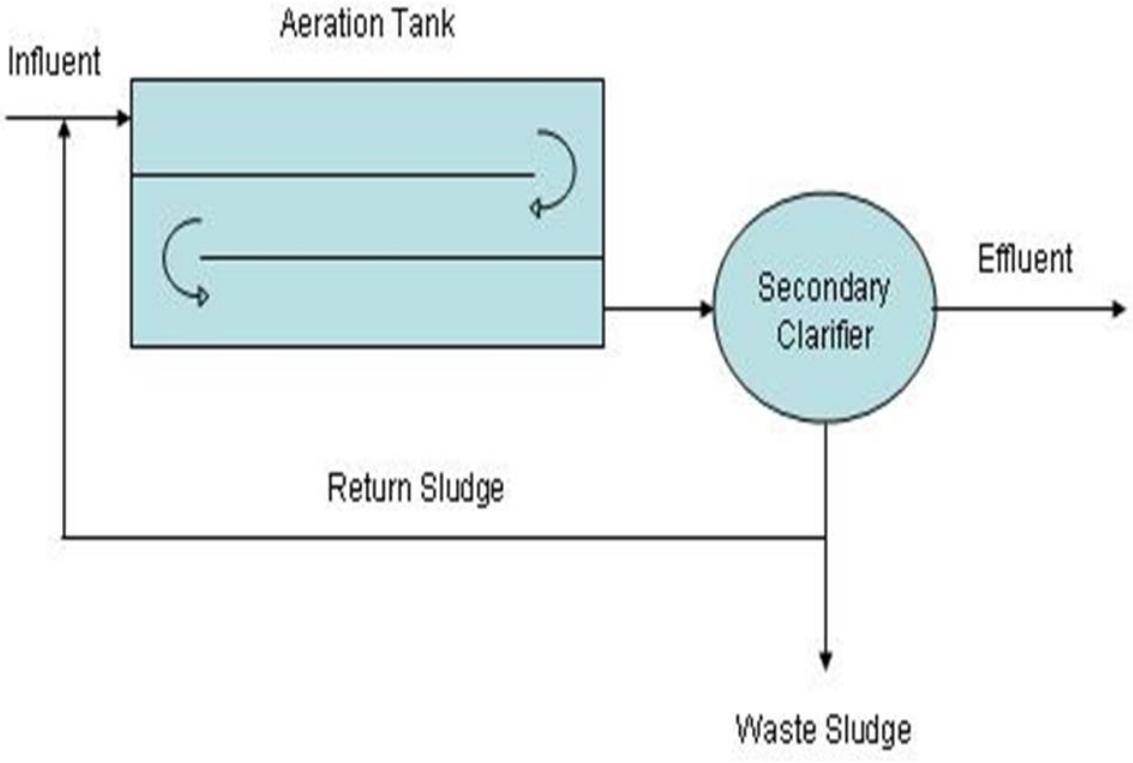
• Phosphorus enters the treatment plant as soluble orthophosphate, soluble polyphosphates, and organically bound phosphorus. Most of the polyphosphates and much of the organically bound phosphorus are converted to more simple orthophosphates during biological treatment. If the influent contains significant polyphosphates and/or organically bound phosphorus, locating chemical treatment after biological processes would be more efficient and achieve lower effluent levels.

• The removal of carbonate alkalinity and phosphorus by lime prior to biological treatment can have a negative impact on nitrification processes (WEF and ASCE, 1998). Also, removing phosphorus to very low concentrations upstream of denitrification filters can negatively affect the denitrification process. Previous studies showed that the hydroxide alkalinity can be balanced by the hydrogen ions produced during nitrification.

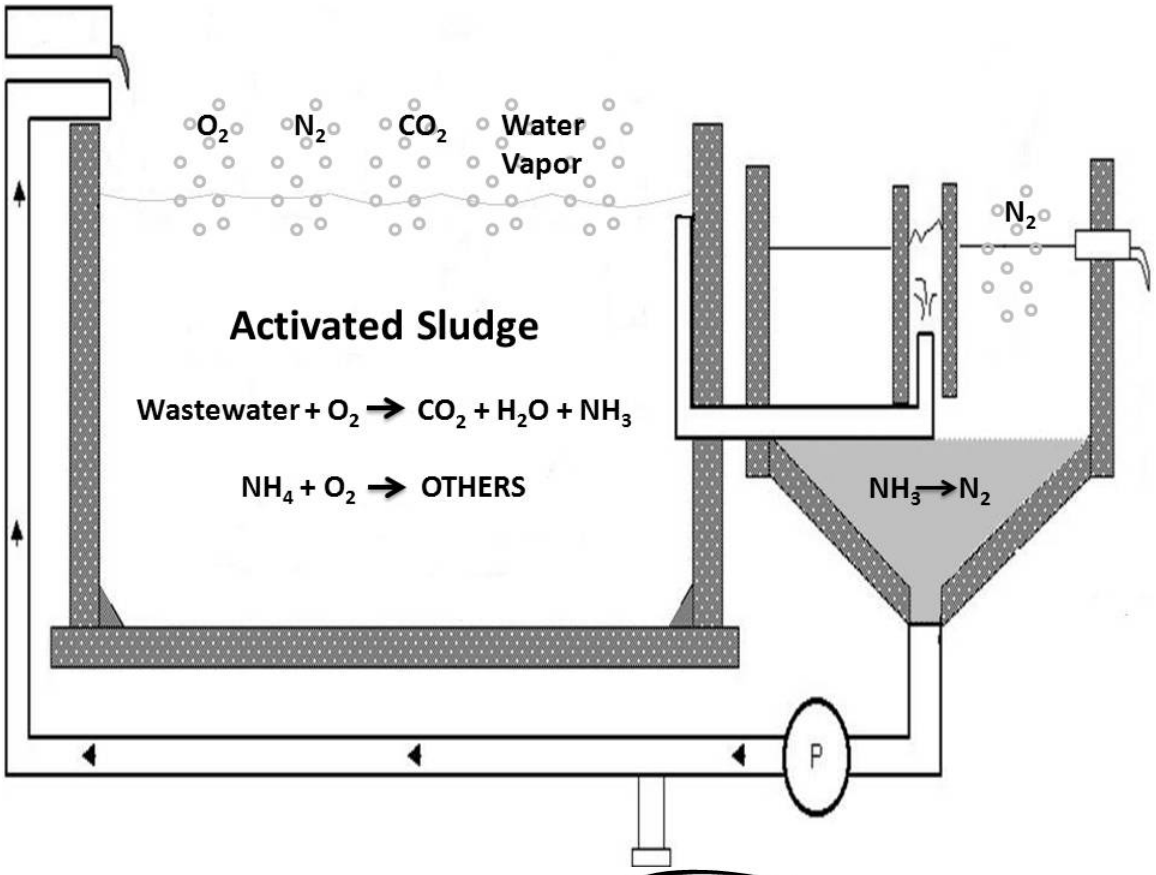
• Sludge recalcification can be used to achieve high removal efficiencies using lime in tertiary treatment. One potential advantage to adding chemicals during primary treatment instead of tertiary treatment is reduced capital costs and space requirements as a result of removing additional BOD and TSS and reducing the load to downstream processes, thereby reducing the size of the subsequent activated sludge basins and the amount of oxygen transfer needed.

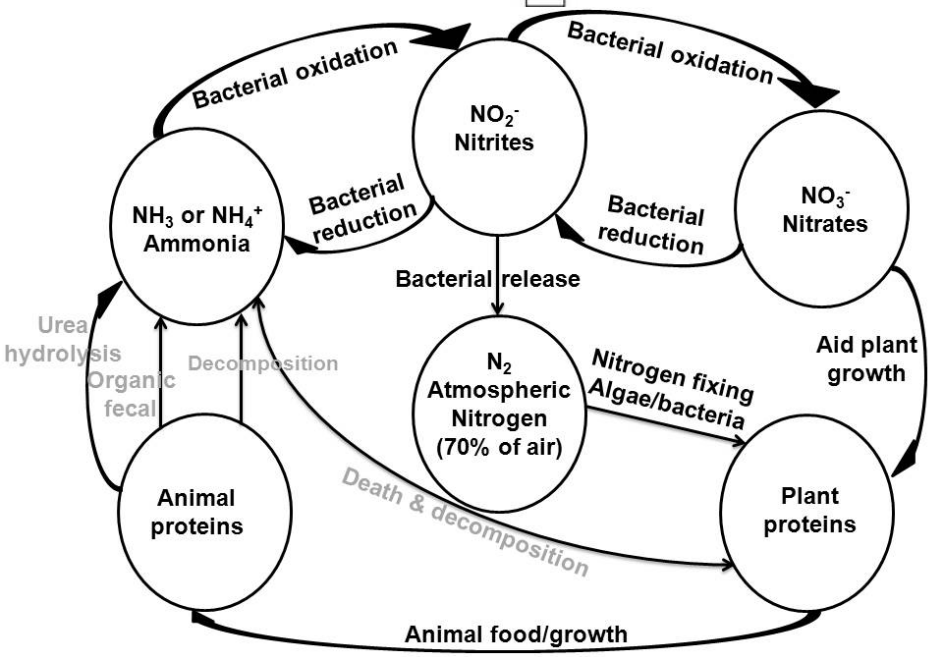
Chemicals should be well mixed with the wastewater to ensure reaction with soluble phosphates and formation of precipitates. Chemicals may either be mixed in separate tanks or can be added at a point in the process where mixing already occurs. Bench-scale and pilot scale tests are often used to determine the correct mixing rate for a given composition of wastewater and chemicals used, including polymer (USEPA, 1999a).



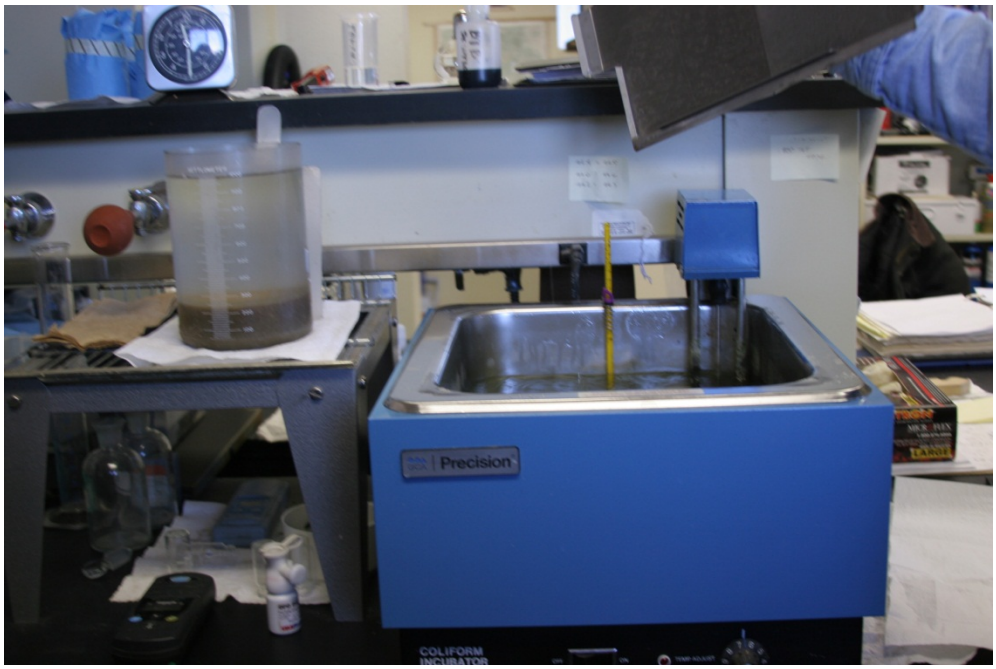


Plug Flow Activated Sludge Process

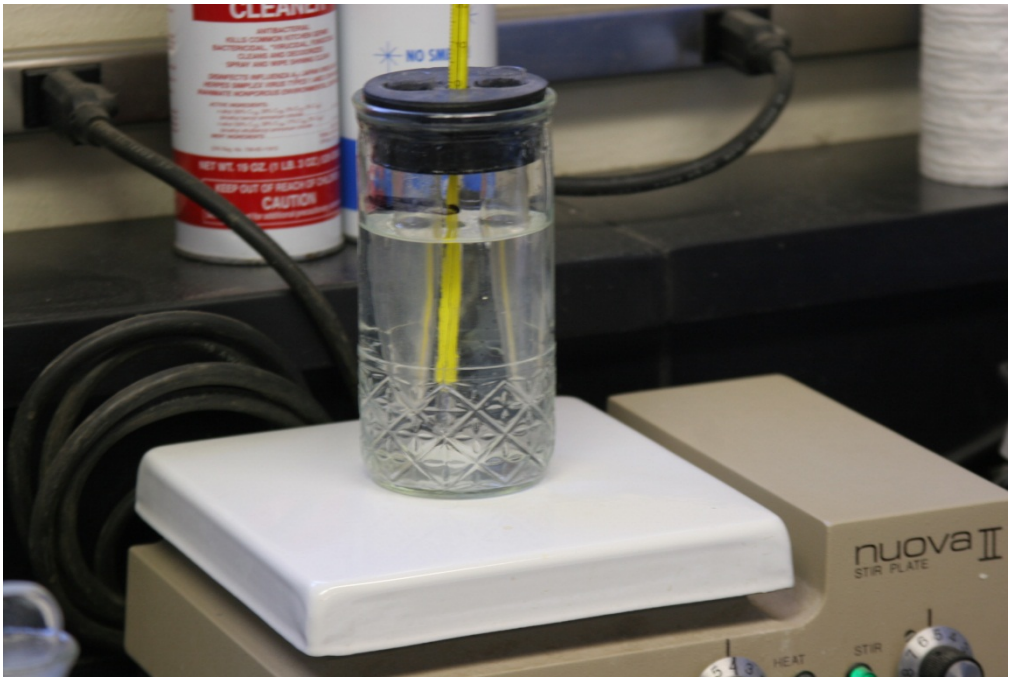




NITROGEN CYCLE



Here is an incubator for the coliform test. The operator will place the sample in this device for 24 to 48 hours depending on the desired results. There are several different methods to calculate coliform bacteria. This is an older true and tested method.



This glass bottle is used for quality control (QA/QC) for bacteria samples tubes.

Advanced Solids Separation Processes

The effectiveness of phosphorus removal by chemical addition is highly dependent on the solids separation process following chemical precipitation. Direct addition of metal salts to activated sludge processes followed by conventional clarification can typically remove TP to effluent levels between 0.5 and 1.0 mg/L (Bott et al., 2007). Tertiary processes (post-secondary treatment) can be used to remove phosphorus to very low (< 0.1 mg/L) concentrations. For example, Reardon (2005) reports that four WWTP with tertiary clarifiers achieved TP levels of between 0.032 and 0.62 mg/L. Two common tertiary processes are clarification and effluent filtration. These approaches can be used separately or in combination. The next section presents a detailed discussion of effluent filtration. Advances in tertiary clarification processes are discussed below.

The types of clarifiers used for tertiary processes include conventional, one or two-stage lime, solids-contact, high-rate, and ballasted high-rate (BHRC). Several patented BHRC using different types of ballast such as recycled sludge, microsand, and magnetic ballast (USEPA, 2008a) have been developed in recent years. The advantages of high-rate clarification are that the clarifiers have a smaller footprint and are able to treat larger quantities of wastewater in a shorter period of time. In addition, as an add-on during wet weather, they can help prevent sanitary sewer overflows (SSOs) and combined sewer overflows (CSOs).

The following patented processes are examples of high rate clarification including performance estimates:

• DensaDeg® uses a coagulant in a rapid mix basin to destabilize suspended solids. The water flows into a second tank where polymer (for aiding flocculation) and sludge are added. The sludge acts as the “seed” for formation of high density floc. This floc is removed in settling tubes (USEPA, 2008). The main advantages of this process are a smaller footprint and denser sludge which is easier to dewater. Pilot testing for City of Fort Worth, Texas found a phosphorus removal rate of 88-95% for DensaDeg® (USEPA, 2003).

• Actiflo® uses a coagulant in a rapid mix basin to destabilize suspended solids. The water flows to a second tank where polymer (for aiding flocculation) and microsand are added. Microsand provides a large surface onto which suspended solids attach, creating a dense floc that settles out quickly. Clarification is assisted by lamella settling. Product pilot testing in Fort Worth, Texas showed a phosphorus removal efficiency of 92-96% for Actiflo®(USEPA, 2003).

• The CoMag process uses the addition of magnetic ballast with metal salts to promote floc formation. Settling is followed by high gradient magnetic separation for effluent polishing and recovery of the magnetic ballast (USEPA, 2008a). CoMag is currently in operation at a 4.0 million gallons per day (MGD) wastewater treatment plant in Concord, Massachusetts. The vendor has guaranteed an effluent phosphorus concentration not to exceed 0.05 mg/L (EPA Region 10, 2007).

Other Design and Operational Issues

Phosphorus removal by chemical addition is limited to the soluble phosphates in the waste stream. Organically bound phosphorus and polyphosphates will not be removed by chemical treatment unless they are coagulated with the chemicals and removed in the sludge. Chemicals can be added after biological treatment to capitalize on the conversion of polyphosphates and organically bound phosphorus to phosphates by microorganisms in activated sludge.

The success of phosphorus removal by chemical addition depends on proper instrumentation and control. Dosage control typically takes the form of manual operation (for small systems), adjustments based on automatic flow measurements, or the more advanced on-line analyzers with computer-assisted dosage control.

Chemical properties of any water used for making solutions should be considered – tap water high in suspended solids could cause sludge to form when mixed with coagulants (WEF and ASCE, 1998) and could lead to clogging of chemical feed lines. Smith et al. (2008) found that factors such as pH, complexation, mixing, and the coagulant used can limit the removal of phosphorus, especially in the range of <0.1 mg/L.

Impacts on Sludge Handling and Production
Sludge handling and production is generally considered to be one of the main downsides of chemical addition. Chemical precipitation methods always produce additional solids due to generation of metal- or calcium- phosphate precipitates and additional suspended solids (WEF and ASCE, 1998). Chemically treated sludge has a higher inorganic content compared to primary and activated sludge and will increase the required size of aerobic and anaerobic digesters. Additional sludge production can be estimated using reaction equations. The use of metal salts can result in increased inorganic salts (salinity) in the sludge and in the effluent.

Salinity can create problems when biosolids are land applied or when the effluent is returned to existing water supply reservoirs. Biological phosphorus removal was developed in South Africa due to the high rate of indirect recycling of wastewater effluent which led to excessive total dissolved solids (TDS) during dry periods. High total salts can reduce germination rates for crops and negatively affect the soil structure.

Lime traditionally produces a higher sludge volume compared to metal salts because of its reaction with natural alkalinity. An advantage of lime sludge is that some stabilization can occur due to the high pH levels required. One disadvantage is that lime can cause scaling in mechanical thickening and dewatering systems. There are also differences in the amount and characteristics of sludge generated by alum versus ferric salts. Although alum tends to produce less sludge than do ferric salts, alum sludge can be more difficult to concentrate and dewater compared to ferric sludge.

Biological Nitrogen Removal
This section provides an overview of the principles behind biological nitrogen removal and describes the common design configurations in use today. It identifies key operational and design issues (including impacts on sludge handling and production), provides general guidelines on process selection, and summarizes ongoing research efforts in this area. Process configurations that are designed to remove both nitrogen and phosphorus are described latter.

Principles
In wastewater treatment, nitrogen removal occurs in two sequential processes: nitrification and denitrification. An overview of each process is provided below.

Nitrification
Nitrification is an aerobic process in which autotrophic bacteria oxidize ammonia or nitrite for energy production. Nitrification is normally a two-step aerobic biological process for the oxidation of ammonia to nitrate. Ammonia-nitrogen (NH3-N) is first converted to nitrite (NO2 -) by ammonia oxidizing bacteria (AOB). The nitrite produced is then converted to nitrate (NO3-) by nitrite oxidizing bacteria (NOB). Both reactions usually occur in the same process unit at a wastewater treatment plant (e.g. activated sludge mixed liquor or fixed film biofilm).

The group of AOB most associated with nitrification is the *Nitrosomonas* genus, although other AOB such as *Nitrosococcus* and *Nitrosospira* can contribute to the process. *Nitrobacter* are the NOB most associated with the second step, although other bacteria including *Nitrospina*, *Nitrococcus*, and *Nitrospira* have been found to also oxidize nitrite (Tchobanoglous et al., 2003; USEPA, 2007c).

AOB and NOB are classified as autotrophic bacteria because they derive energy from the oxidation of reduced inorganic compounds (in this case, nitrogenous compounds) and use inorganic carbon (CO2) as a food source. Nitrifying bacteria require a significant amount of oxygen to complete the reactions, produce a small amount of biomass, and cause destruction of alkalinity through the consumption of carbon dioxide and production of hydrogen ions. For each gram (g) of NH3-N converted to nitrate, 4.57 g of oxygen are used, 0.16 g of new cells are formed, 7.14 g of alkalinity are removed, and 0.08 g of inorganic carbon are utilized in formation of new cells (Tchobanoglous et al., 2003).

Nitrifying bacteria grow slower and have much lower yields as a function of substrate consumed, compared to the heterotrophic bacteria in biological treatment processes. The maximum specific growth rate of the nitrifying bacteria is 10 to 20 times less than the maximum specific growth rate of heterotrophic bacteria responsible for oxidation of carbonaceous organic compounds in wastewater treatment. Thus, the nitrification process needs a significantly higher SRT to work compared to conventional activated sludge processes. The SRT needed for nitrification in an activated sludge process is a function of the maximum specific growth rate (which is related to temperature), the reactor dissolved oxygen concentration, and pH. Nitrification rates decline as the DO concentration decreases below 3.0 mg/L and the pH decreases below 7.0 mg/L. With sufficient DO and adequate pH, typical nitrification design SRTs range from 10 to 20 days at 10^{o}C and 4 to 7 days at 20^{o}C (Randall et al.,1992).

Denitrification

In municipal and industrial wastewater treatment processes, denitrification is the biological reduction of nitrate or nitrite to nitrogen gas (N2) as indicated by equation below.

NO → NO → NO → N O → N (5-1)

It is accomplished by a variety of common heterotrophic microorganisms that are normally present in aerobic biological processes. Most are facultative aerobic bacteria with the ability to use elemental oxygen, nitrate, or nitrite as their terminal electron acceptors for the oxidation of organic material.

Heterotrophic bacteria capable of denitrification include the following genera: *Achromobacter, Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter, Bacillus, Chromobacterium, Corynebacterium, Flavobacterium, Hypomicrobium, Moraxella, Nesseria, Paracoccus, Propionibacteria, Pseudomonas, Rhizobium, Rhodopseudmonas, Spirillum and Vibrio* (Tchobanoglous et al., 2003).

Recent research has shown that nitrite reduction is accomplished by a much more specialized group of heterotrophic bacteria than those performing the conversion of nitrate to nitrite (Katehis, 2007).

Denitrification by heterotrophic nitrifying bacteria and by autotrophic bacteria has also been observed. An example of a heterotrophic nitrifying bacteria that can denitrify is *Parococcus pantotropha*, which obtains energy by nitrate or nitrite reduction while oxidizing ammonia under aerobic conditions. A readily available carbon source, such as acetate, is needed (Robertson and Kuenen, 1990). The conditions required for this form of denitrification are not practical in biological wastewater treatment.

An autotrophic denitrifying bacteria of practical significance in wastewater treatment is that in the Anammox process used to remove nitrogen in return streams from anaerobic digestion sludge dewatering filtrate or centrate. These bacteria have been identified as a member of bacteria in the order *Planctomycetales* (Strous et al, 1999). Under anaerobic conditions, ammonia is oxidized with the reduction of nitrite with the final product as nitrogen gas. The reaction is best accomplished at temperatures above 25°C and they are slow growing organisms.

Facultative denitrifying bacteria will preferentially use oxygen instead of nitrate. In the absence of oxygen, however, they will carry out nitrite and/or nitrate reduction. Microbiologists generally use the term anaerobic to describe biological reactions in the absence of oxygen. To distinguish anaerobic conditions for which the biological activity occurs mainly with nitrate or nitrite as the electron acceptor, the term “anoxic” has been applied.

Although oxygen is known to inhibit denitrification, denitrification has been observed in activated sludge and fixed film systems in which the bulk liquid DO concentration is positive. This is due to the establishment of an anoxic zone within the floc or biofilm depth. Hence, a single system can carry out simultaneous nitrification and denitrification. The DO concentration that is possible for simultaneous nitrification and denitrification depends on a number of factors including the mixed liquor concentration, temperature, and substrate loading. The DO concentration above which denitrification is inhibited may vary from 0.10 to 0.50 mg/L (WEF and ASCE, 2006; Tchobanoglous et al., 2003; Barker and Dold, 1997).

The organic carbon source for denitrifying bacteria can be in the form of:
- Soluble degradable organics in the influent wastewater
- Soluble organic material produced by hydrolysis of influent particulate material
- Organic matter released during biomass endogenous decay

A general rule of thumb is that 4 g of wastewater influent BOD is needed per g of NO3-N to be removed through biological treatment (Tchobanoglous et al., 2003). When denitrification occurs after secondary treatment, there is little BOD remaining so a supplemental carbon source is often needed. The most common exogenous carbon source in use is methanol; however, due to issues regarding its safety, cost, and availability, some wastewater systems are using alternative carbon sources such as acetic acid, ethanol, sugar, glycerol, and proprietary solutions depending on the needs of their particular facility (deBarbadillo et al., 2008).

Biological denitrification reactions produce alkalinity and heterotrophic biomass. Based on the stoichiometry of the reactions, denitrification will produce a 3.57 mg/L of alkalinity as CaCO3 for each mg/L of $NO3^{-}$−N consumed. Heterotrophic biomass produced can be estimated as 0.4 g volatile suspended solids (VSS) produced for every gram of COD consumed. Growth kinetics for denitrifiers are dependent on a number of factors including carbon substrate type and concentration, DO concentration, alkalinity, pH, and temperature, with carbon source being the most important.

Current Configurations
Biological nitrogen removal can be accomplished by a variety of treatment configurations using suspended growth, attached growth, or combined systems. In the past, some WWTPs were required to only remove ammonia-nitrogen in wastewater to reduce toxicity to aquatic organisms with no limits on nitrate or total nitrogen. However, most treatment plants are now required to remove nitrogen because both ammonia-nitrogen and nitrate-nitrogen can stimulate algae and phytoplankton growth and lead to eutrophication of U.S. waterways. For biological nitrogen removal, it is essential that nitrification occur first followed by denitrification.

Biological Nitrogen Removal Process Configurations
Biological nitrogen removal systems achieve nitrification and denitrification along with BOD reduction in bioreactors followed by secondary clarification. Processes can be either suspended growth or hybrid systems that use a combination of attached growth (biofilms) and suspended growth technologies. Configurations within each of these classifications are discussed below. Note that biological processes that removal both nitrogen and phosphorus are discussed later in this manual.

Suspended Growth Systems Modified Ludzck Ettinger (MLE) process
The most common nitrogen removal process used at WWTPs is the Modified Ludzck Ettinger (MLE) process, which is considered a pre-denitrification, single sludge system. The process includes an initial anoxic zone, followed by an aerobic zone. In the anoxic zone, nitrate produced in the aerobic zone is reduced to nitrogen gas. This process uses some of the BOD in the incoming waste. Nitrification occurs in the aerobic zone along with the removal of most of the remaining BOD. At the end of the aerobic zone, pumps recycle the nitrate-rich mixed liquor to the anoxic zone for denitrification.

Total nitrogen removal for the MLE process is typically 80 percent, and the process achieves total effluent nitrogen concentrations ranging from approximately 5 to 8 mg/L with internal nitrate recycle ratios of 2 to 4 based on the influent flowrate (2-4Q).

Four-Stage Bardenpho Process
The four-stage Bardenpho process builds on the MLE process, with the first two stages being identical to the MLE system (anoxic zone followed by an aeration zone with a nitrate-rich recycle from the aeration to the anoxic zone). The third stage is a secondary anoxic zone to provide denitrification to the portion of the flow that is not recycled to the primary anoxic zone. Methanol or another carbon source can be added to this zone to enhance denitrification. The fourth and final zone is a re-aeration zone that serves to strip any nitrogen gas and increase the DO concentration before clarification. Some configurations have used an oxidation ditch instead of the first two stages. This process can achieve effluent TN levels of 3 to 5 mg/L.

Sequencing Batch Reactors
Sequencing batch reactors (SBRs) are fill and draw batch systems in which all treatment steps are performed in sequence for a discreet volume of water in a single or set of reactor basins.

SBRs use four basic phases for most systems:
Fill: water is added to the basin and is aerated and mixed
React: Biological processes are performed
Settle: All aeration and mixing is turned off and the biomass is allowed to settle
Decant: Clarified effluent is removed and biomass is wasted as necessary

The SBR control system allows it to mimic most other suspended growth processes such as the MLE or Four-Stage Bardenpho system. It typically completes 4 to 6 cycles per day per tank for domestic wastewater. If properly designed and operated, SBRs can achieve about 90 percent removal of nitrogen (WEF and ASCE, 2006).

Oxidation Ditches
Oxidation ditches are looped channels that provide continuous circulation of wastewater and biomass. A number of operating methods and designs have been developed to achieve nitrogen removal, all of which work by cycling the flow within the ditch between aerobic and anoxic conditions. DO can be added to the aerated zone using horizontal brush aerators, diffused aerators with submersible mixers, or vertical shaft aerators (WEF and ASCE, 2006). Patented designs include the NITROX process, Carrousel, and BioDenitro (WERF, 2000a). Many oxidation ditch configurations can achieve simultaneous nitrogen and phosphorus removal.

Step Feed
The step feed biological nitrogen removal process splits the influent flow and directs a portion of it to each of several anoxic zones, with the highest proportion of influent flow going to the first zone and steadily decreasing until the last anoxic zone prior to clarification. The biomass in the later stages are not just treating influent flow but are also used to reduce nitrate from the upstream zones. The step feed system provides flexibility for systems to handle wet-weather events. It can also be compatible with existing conventional “plug flow” activated sludge processes and it does not require the installation of recycle pumps and piping.

Disadvantages include the need to control the DO concentration of aeration zones preceding the downstream anoxic zones and the need to control the flow splitting to the step feed points.

Attached Growth and Hybrid Systems Integrated Fixed-Film Activated Sludge (IFAS)
Integrated fixed-film activated sludge (IFAS) is any suspended growth system (e.g., MLE, Four-Stage Bardenpho) that incorporates an attached growth media within the suspended growth reactor in order to increase the amount of biomass in the basin. IFAS systems have higher treatment rates than suspended growth systems and generate sludge with better settling characteristics. Many types of fixed and floating media are available, including:

• **Rope**: also called looped-cord or strand media. Consists of a polyvinyl chloride-based material woven into rope with loops along the length to provide surface area for the biomass (WERF, 2000b). Proprietary designs include Ringlace, Bioweb, and Biomatrix (USEPA, 2008a).

• **Moving Bed with Sponge:** proprietary products include Captor and Linpor (USEPA, 2008a).

• **Plastic Media:** several types of free-floating plastic media are available from Kaldness. Other media types include fabric mesh (e.g., AccuWeb) and textile material (Cleartec).

Moving-Bed Biofilm Reactor (MBBR)
The moving-bed biofilm reactor (MBBR) is similar to the IFAS system in that it uses plastic media with a large surface area to increase biomass within the biological reactor. The MBBR media is submerged in a completely mixed anoxic or aerobic zone. The plastic media are typically shaped like small cylinders to maximize surface area for biomass growth. The difference between MBBR and IFAS is that MBBR does not incorporate return sludge (WERF, 2000b).

Membrane Bioreactor (MBR)
MBRs are commonly designed for nitrogen removal, using membranes for liquid-solids separation following the anoxic and aerobic zones instead of conventional clarification. Membranes can be submersed in the biological reactor or located in a separate stage or compartment. Low-pressure membranes (ultrafiltration or microfiltration) are commonly used. Systems can be pressure driven or vacuum. All systems use an air scour technique to reduce buildup on the membranes (USEPA, 2007d; USEPA, 2008a).

Membrane materials are either organic polymers or inorganic materials such as ceramics. They are designed in modular units and are typically configured as either hollow fiber bundles or plate membranes (USEPA, 2007d) For biological nutrient removal applications, the design SRTs and design principals for MBR systems are similar to those used for systems with secondary clarifiers.

One of the main differences is that the MBR systems operate at a higher MLSS concentration which results in smaller tanks and smaller space requirements. In addition, membrane separation provides for greatly reduced TSS in the effluent, typically below 1.0 mg/L, and hence slightly greater removal of nitrogen and phosphorus. Operational issues include potential for membrane biofouling and increase pumping costs (USEPA, 2007d; WEF,2005).

Activated Sludge Process Section

Key Terms

Aerobic (AIR-O-bick) a condition in which free or dissolved oxygen is present in the aquatic environment

Aerobic Bacteria – bacteria which will live and reproduce only in an environment containing oxygen.

(aerobes) Oxygen combined chemically, such as in water molecules (H_2O), cannot be used for respiration by aerobes

Anaerobic (AN-air O-bick)- a condition in which ***"free"*** or dissolved oxygen is not present in the aquatic environment.

Anaerobic Bacteria – bacteria that thrive without the presence of oxygen.
(anaerobes)

Saprophytic bacteria – bacteria that break down complex solids to volatile acids.

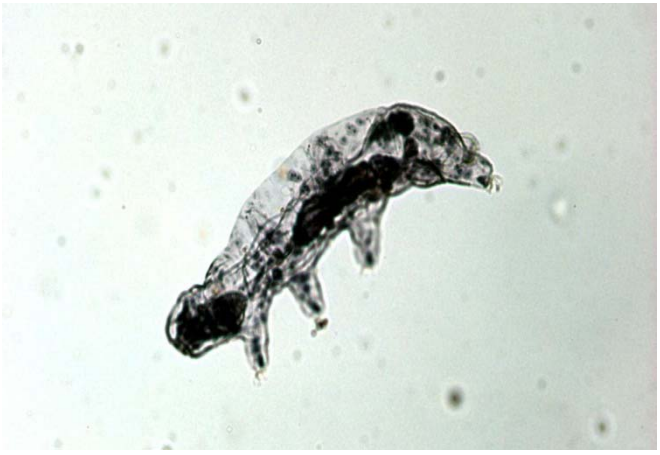
Methane Fermenters – bacteria that break down the volatile acids to methane (CH_4) carbon dioxide (CO_2) and water (H_2O).

Oxidation – the addition of oxygen to an element or compound, or removal of hydrogen or an electron from an element or compound in a chemical reaction. The opposite of reduction.

Reduction – the addition of hydrogen, removal of oxygen or addition of electrons to an element or compound. Under anaerobic conditions in wastewater, sulfur compounds or elemental sulfur are reduced to H_2S or sulfide ions.

Water Bear Tardigrade

This creepy "water bear" has forced scientists to reconsider their definition of what's "alive." When unable to find water, this insect like critter (which is the size of a grain of sand) stops moving, breathing, and eating. Even its cells shut down. Dead as a doornail, right? Wrong! Add water and the critter springs back to life. Scientists have exposed dried-up water bears to extreme heat, bitter cold, and even massive doses of radiation -- and the teeny animals still revived. The key to the critters' survival may be a sugar they produce as they dry out. By coating structures inside and between the critters' cells, the sugar keeps the cells from sticking together and breaking. When water is added, the sugar dissolves -- and the creepy crawlies burst back into action.



Basic System Components of Activated Sludge

In the basic "activated" sludge process, emphasis on *activated***,** the wastewater enters an aerated tank (the dome) where previously developed biological floc particles are brought into contact with the organic matter (foot-long hot dogs) of the wastewater.

The organic matter is a carbon and an energy source for the bug's cell growth and is converted into cell tissue. The oxidized end product is mainly carbon dioxide, CO_2. The substance in the sports dome is referred to as mixed liquor. The stuff in the mixed liquor is suspended solids and consists mostly of microorganisms, suspended matter, and non-biodegradable suspended matter (***MLVSS***).

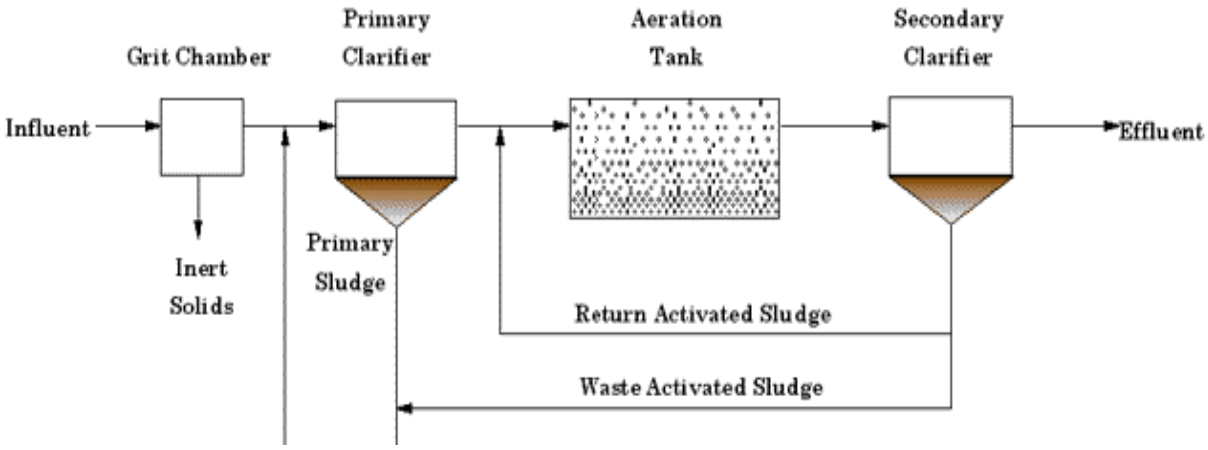
The make-up of the microorganisms are around 70 to 90% organic and 10 to 30% inorganic matter. The makeup of cells varies depending on the chemical composition of the wastewater and the specific characteristics of the organisms in the biological mass. The picture below shows the basic outline of an aeration tank. Just remember that pretreatment is crucial prior to the activated sludge process.

Before we dive into the tank, in the space provided, list three key components of pretreatment (headworks) and how each benefits the process.

1.

2.

3.



Mixed Liquor
Back to the mixed liquor, as it leaves the aeration tank, it usually goes to a clarifier to separate the suspended solids (**SS**) from the treated wastewater. The concentrated biological solids are then recycled back to the aeration tank, as returned activated sludge (**RAS**), to maintain a concentrated population of bugs (the team players) to treat the wastewater.

Before we start the game, we need to make sure we have a stadium and all components are in place and operating properly. In the space provided, define the following terms:
See Glossary in Rear.

Anaerobic:

Aerobic:

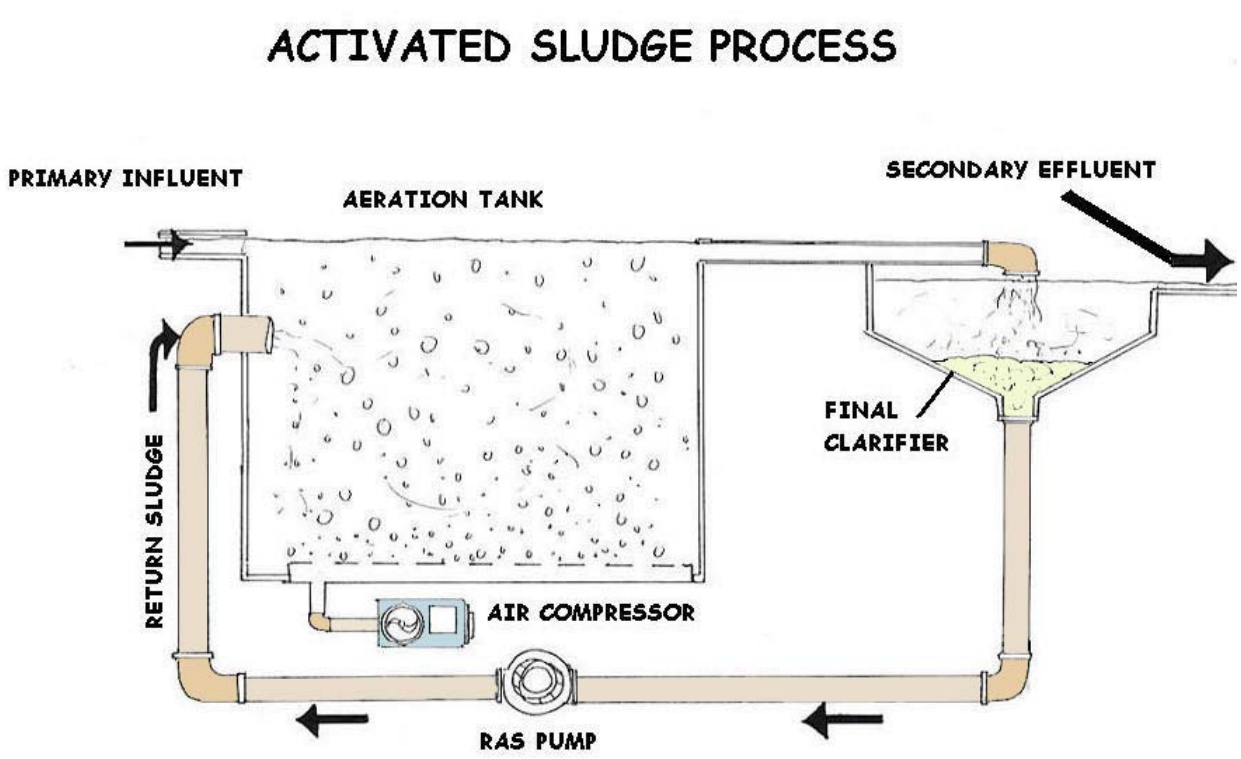
DO:

BOD:

COD:

Process Design

Let's first look at the different aeration tank designs and how they function. We will focus on the following:

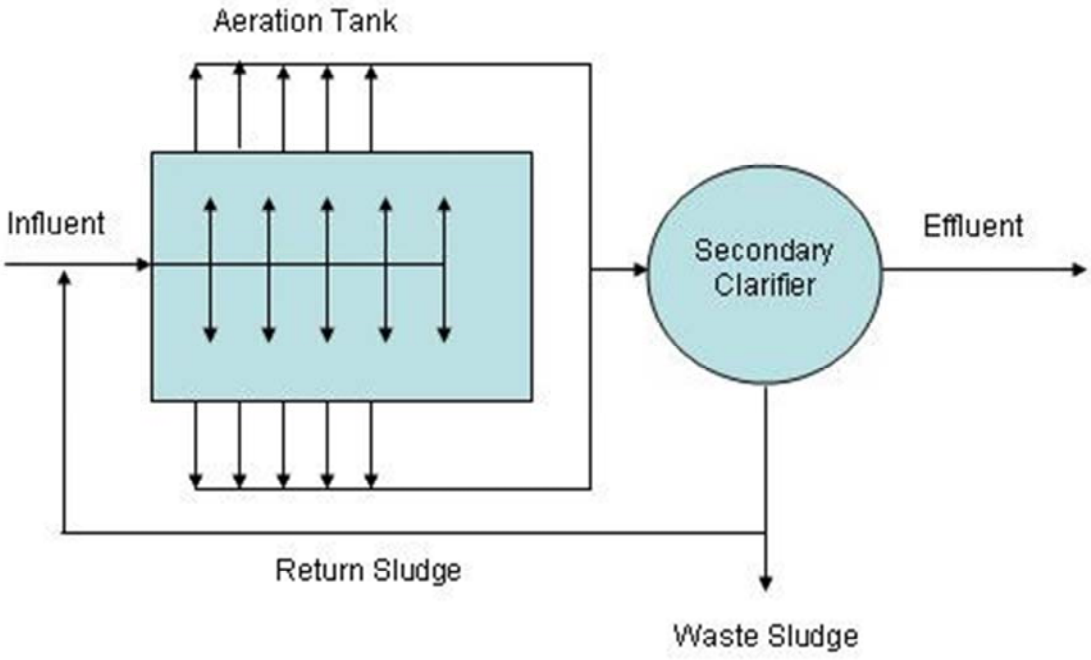


Complete Mix Activated Sludge Process

In a complete mix activated sludge process, the mixed liquor is similar throughout the aeration tank. The operating characteristics measured in terms of solids, oxygen uptake rate (**OUR**), MLSS, and soluble BOD 5 concentration are identical throughout the tank.

Because the entire tank contents are the same quality as the tank effluent, there is a very low level of food available at any time to a large mass of microorganisms.

This is the major reason why the complete mix modification can handle surges in the organic loading without producing a change in effluent quality. The type of air supply used could be either diffused air or a mechanical aerator. Complete mix process may be resistant to shock loads but is susceptible to filamentous growths.

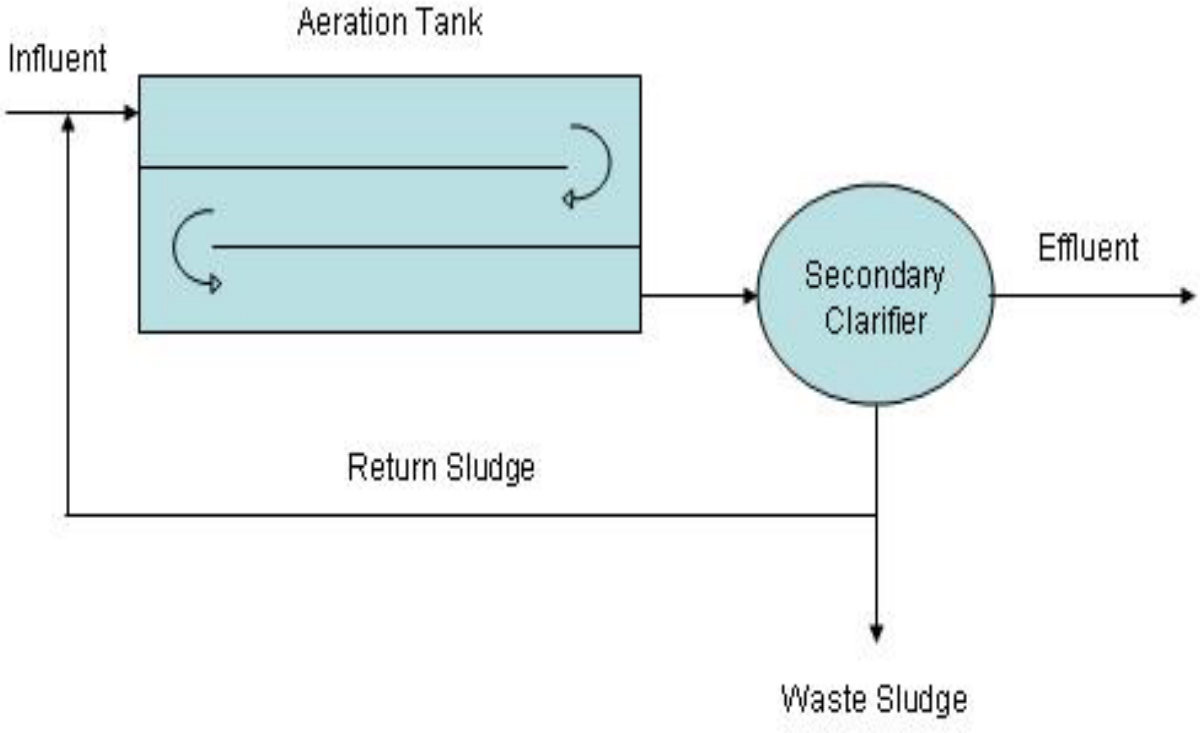


Plug Flow Activated Sludge Process

Plug flow tanks are the oldest and most common form of aeration tank. They were designed to meet the mixing and gas transfer requirements of diffused aeration systems. One characteristic of the plug flow configuration is a very high organic loading on the MLSS in the initial part of the tank. The loading is then reduced and the organic material in the raw wastewater is oxidized.

At the end of the tank, depending on detention time, the oxygen consumption may primarily be the result of endogenous respiration or nitrification, which we will talk more about later on. The same characteristics are present when the aeration tank is partitioned into a series of compartments.

Each compartment must have the oxygen supply and design to meet the individual compartment needs. Plug flow configurations have the ability to avoid **"*bleed through,*"** the passage of untreated organics during peak flow. These configurations are often preferred when high effluent DO's are sought because only a small section of the tank will operate at a high DO. In a complete mix configuration, the entire tank must operate at the elevated DO.



Contact Stabilization Activated Sludge Process

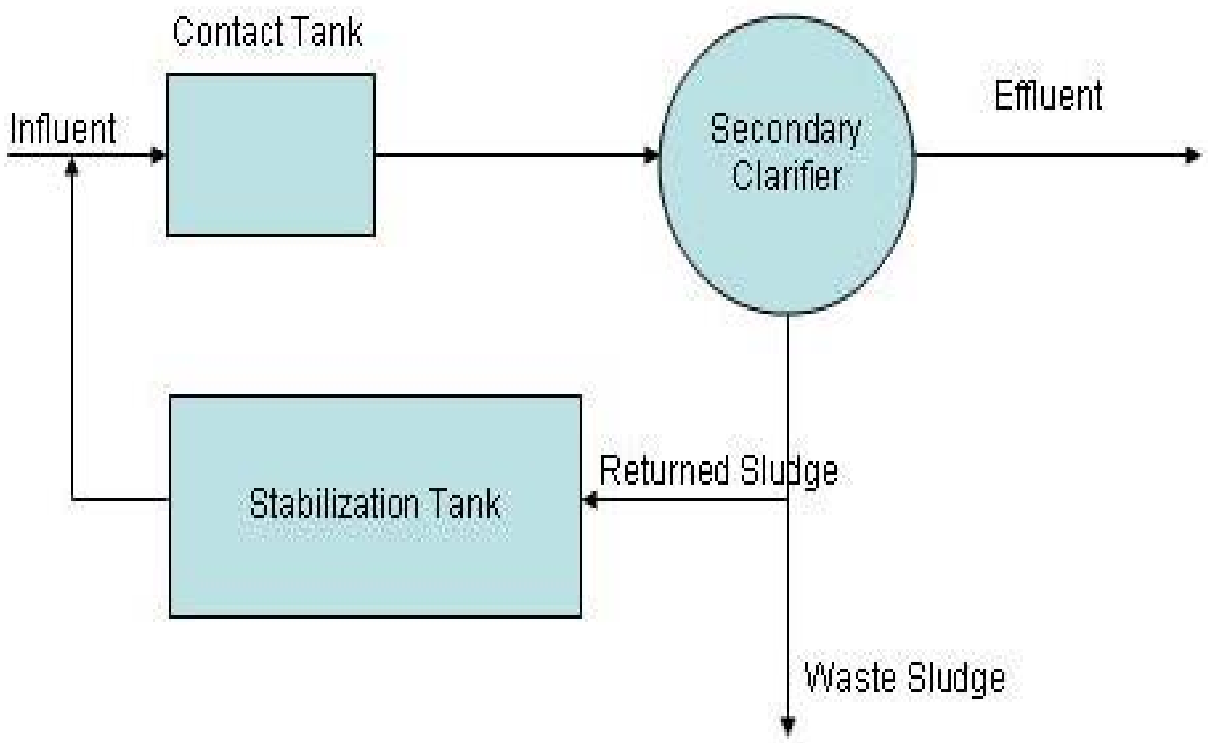
Contact stabilization activated sludge is both a process and a specific tank configuration. The contact stabilization encompasses a short-term contact tank, secondary clarifier, and a sludge stabilization tank with about six times the detention time used in the contact tank.

Contact stabilization is best for smaller flows in which the MCRT desired is quite long.

Therefore, aerating return sludge can reduce tank requirements by as much as 30 to 40 % versus that required in an extended aeration system. The volumes for the contact and stabilization tanks are often equal in size and secondary influent arrangements.

What does this all mean?

They can be operated either in parallel as an extended aeration facility or as a contact stabilization unit. This flexibility makes them suitable for future expansion to conventional activated sludge, without increasing the aeration tank, by merely adding more clarification capacity.



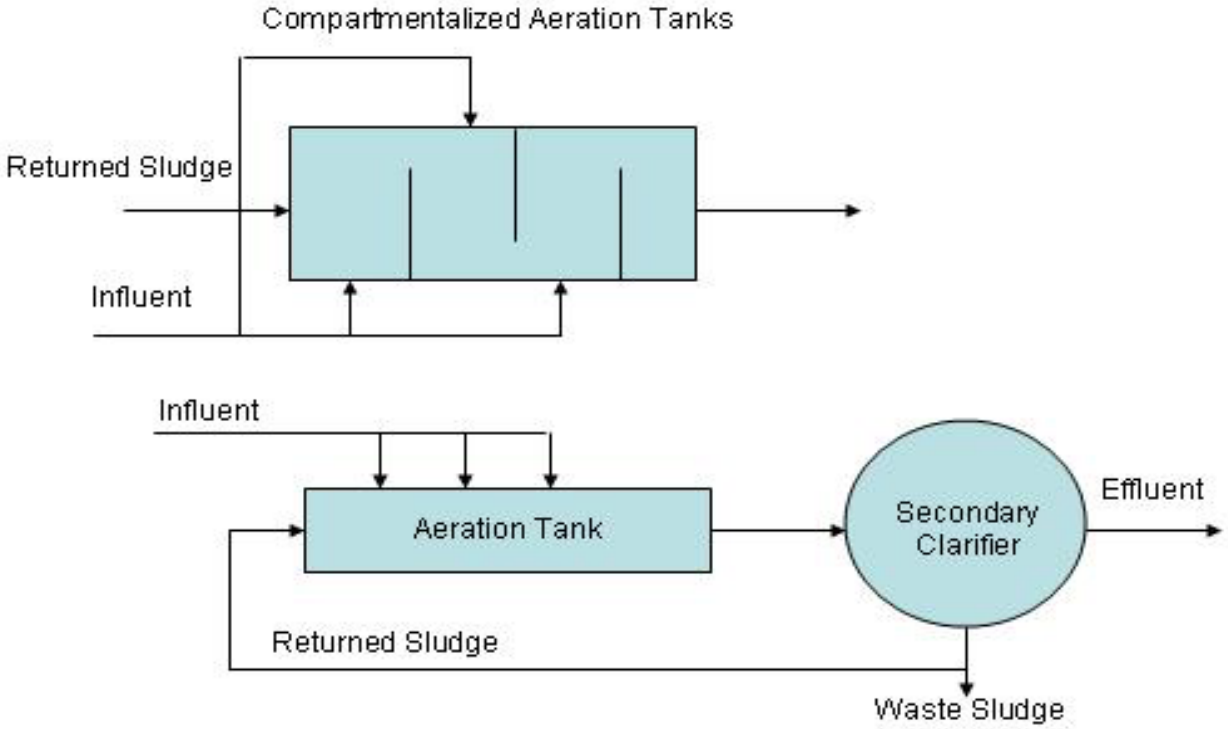
Step Feed Activated Sludge Process

Step feed is a modification of the plug flow configuration in which the secondary influent is fed at two or more points along the length of the aeration tank.

With this arrangement, oxygen uptake requirements are relatively even and the need for tapered aeration is eliminated.

Step feed configurations generally use diffused aeration equipment. The step feed tank may be either the long rectangular or the folded design. Secondary influent flow is added at two or more points to the aeration tank, usually in the first 50 to 75% of the length.

It is also possible to use the same process approach by compartmentalizing the tank and directing flow lengthwise through the compartments. Usually, the last compartment does not receive any raw waste.



Extended Aeration Activated Sludge Process

The extended aeration process uses the same flow scheme as the complete mix or plug flow processes but retains the wastewater in the aeration tank for 18 hours or more.

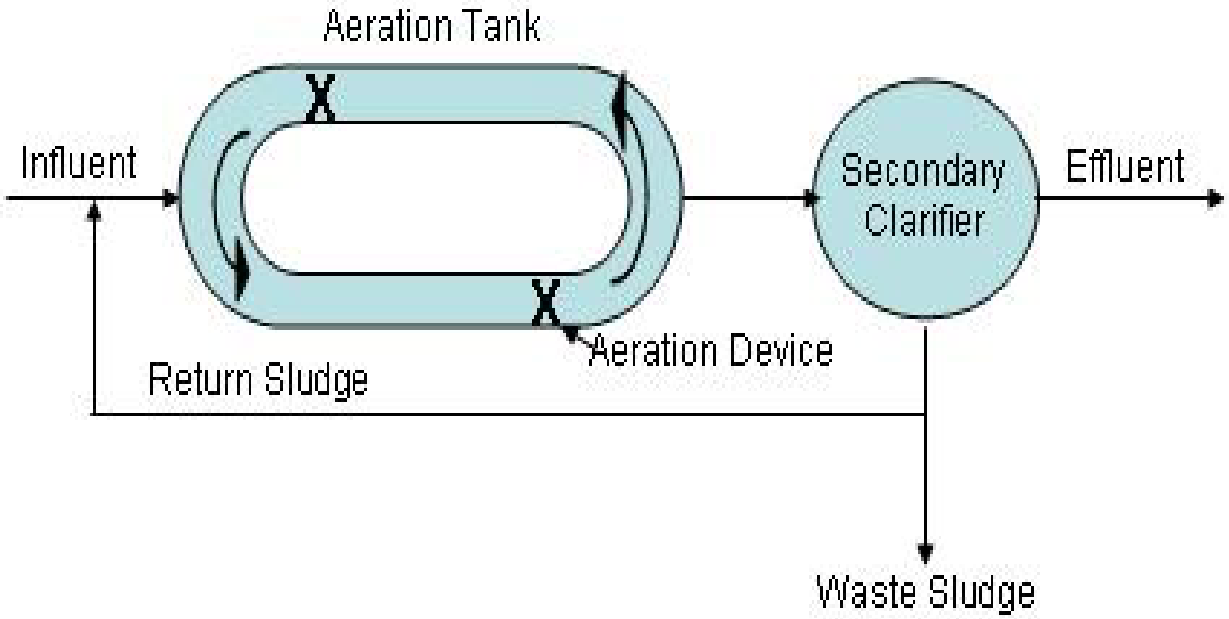
This process operates at a high MCRT (low F/M), resulting in a condition where there is not enough food in the system to support all of the microorganisms present. The microorganisms therefore compete very actively for the remaining food and even use their own cell structure for food.

This highly competitive situation results in a highly treated effluent with low sludge production. (Many extended aeration systems do not have primary clarifiers and they are package plants used by small communities.)

The main disadvantages of this system are the large oxygen requirements per unit of waste entering the plant and the large tank volume needed to hold the wastes for the extended period.

Oxidation Ditch Activated Sludge Process
The oxidation ditch is a variation of the extended aeration process. The wastewater is pumped around a circular or oval pathway by a mechanical aerator/pumping device at one or more points along the flow pathway. In the aeration tank, the mixed liquor velocity is maintained between 0.8 and 1.2 fps in the channel to prevent solids from settling.

Oxidation ditches use mechanical brush disk aerators, surface aerators, and jet aerator devices to aerate and pump the liquid flow. Combination diffused aeration and pumping devices are commonly used in Europe.



High Purity Oxygen Activated Sludge Process

The most common high purity oxygen activated sludge process uses a covered and staged aeration tank configuration. The wastewater, return sludge, and oxygen feed gas enter the first stage of this system and flow concurrently through the tank.

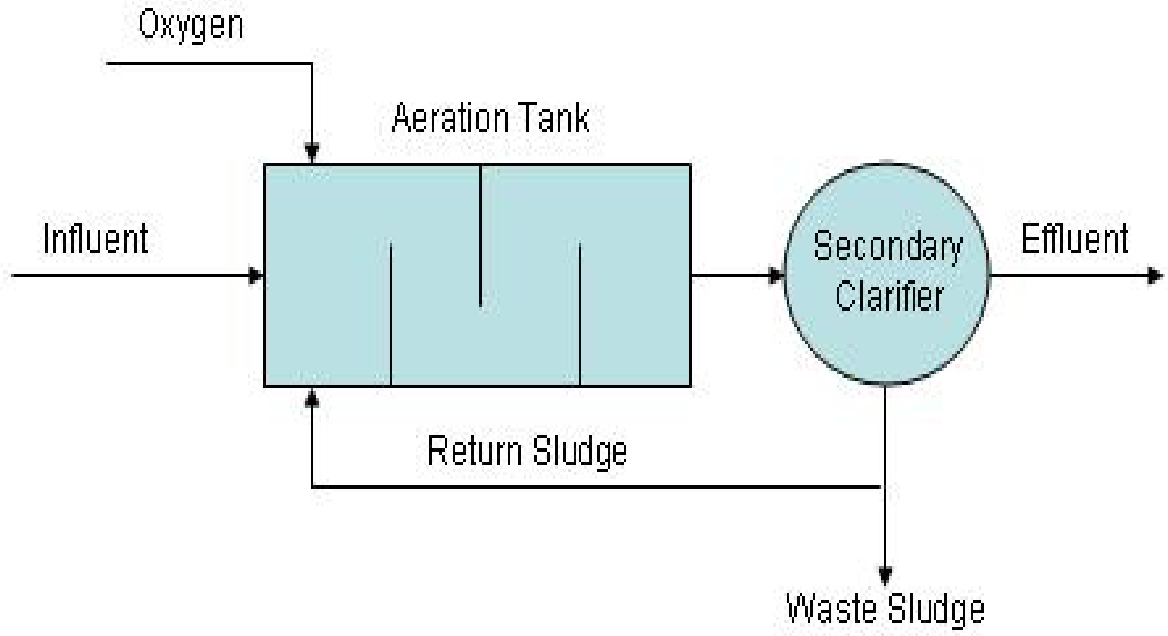
The tanks in this system are covered to retain the oxygen gas and permit a high degree of oxygen use. A prime advantage of the staged reactor configuration of the oxygenation system is the system's ability to match the biological uptake rate with the available oxygen gas purity.

The dissolution of oxygen and the mixing of the biological solids within each stage of the system are accomplished with either surface aeration devices or with submerged turbine-aeration systems. The selection of either of these two types of dissolution systems largely depends on the aeration tank geometry selected.

The particular configuration of oxygenation tank selected for a given system, that is, size of each stage, number of stages per aeration tank, and number of parallel aeration tanks, is determined by several parameters including waste characteristics, plant size, land availability, and treatment requirements.

Aside from the aeration tank, the other key factor in an oxygen activated sludge system is the oxygen gas source. There are three sources of oxygen supply: liquid oxygen storage, cryogenic oxygen generation, and pressure-swing adsorption generation.

The first of these requires no mechanical equipment other than a storage tank that is replenished by trucked-in liquid oxygen. This method is economically feasible for small (less than 4 mgd) or temporary installations.



Aeration Section

There are several designs and applications for aerators:

- Diffused Aerators
- Mechanical Surface Aerators
- Submerged Turbine Aerators

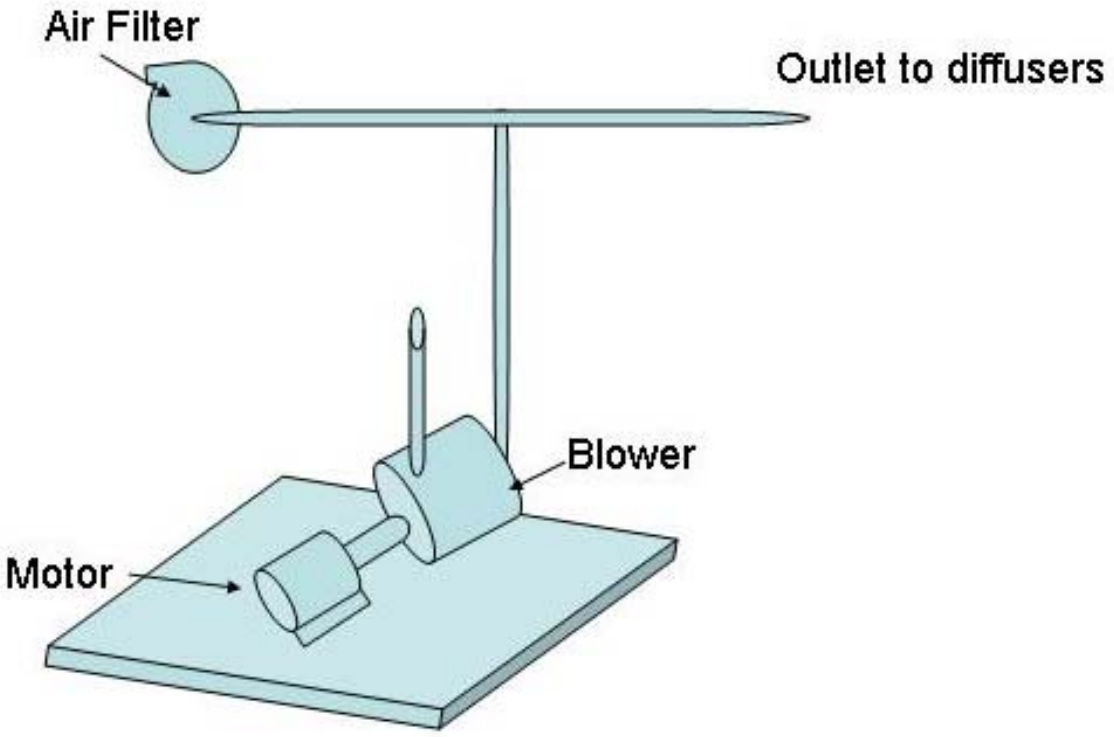
The two most common types of aeration systems are subsurface diffusion and mechanical aeration. Diffused air systems have been around longer than you.

Opened tubes were used or perforated pipes located at the bottom of aeration tanks. But a more efficient process was desired, born to the process, porous plate diffusers. In the diffused air system, compressed air is introduced near the bottom of the tank. Let's look at the definition for diffused aeration:

"The injection of a gas, air or oxygen, below a liquid surface."

There are a variety of hybrid air diffusion systems used in the process; we will focus on the basic components.

The following diagram highlights the main parts of the diffused aeration system.

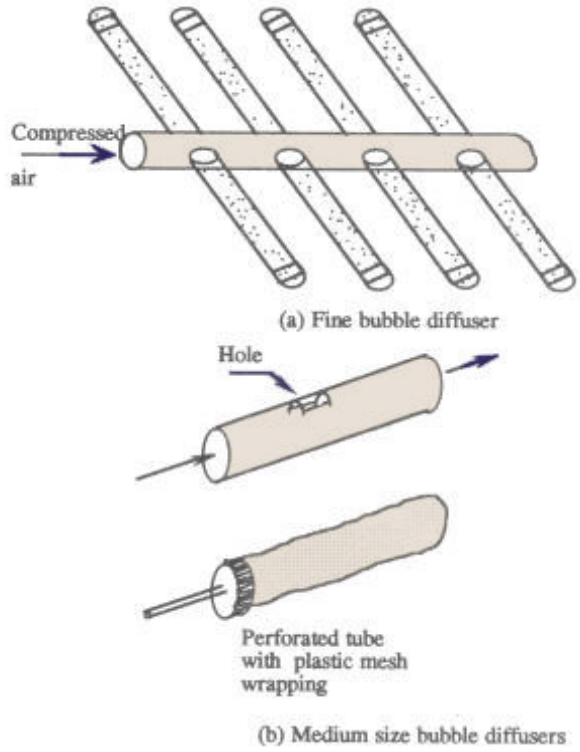


Here is a rare and up-close view of non-porous diffuser heads. Notice the heads that are missing in the bottom photograph.

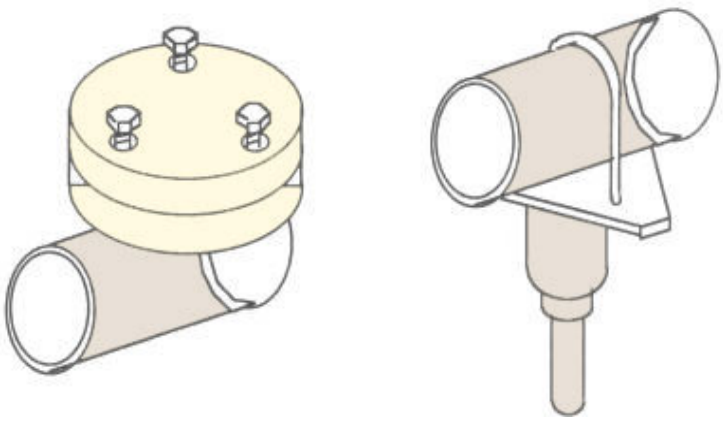
Aeration

The aerated systems described above need an oxygen supply. Depending on the characteristics of the process, different designs may be used. The oxygen can be supplied to the activated sludge by either diffused aeration, by turbine agitation, by static aerators, or by surface coarse or large bubble diffusers. The last two are also used in lagoon systems.

The diffused aeration systems are also divided into fine bubble, medium and coarse or large bubble diffusers. The fine bubble diffusers are built of porous materials (grains of pure silica or aluminum oxide are bonded ceramically or by resins) which provide very small bubbles of high surface area that favor the oxygen transfer from the air to the wastewater. The medium bubble diffusers are perforated pipes or tubes wrapped with plastic or woven fabric. The coarse or large bubble diffusers can be orifice devices of various types, some of which are designed to be non-clogging.

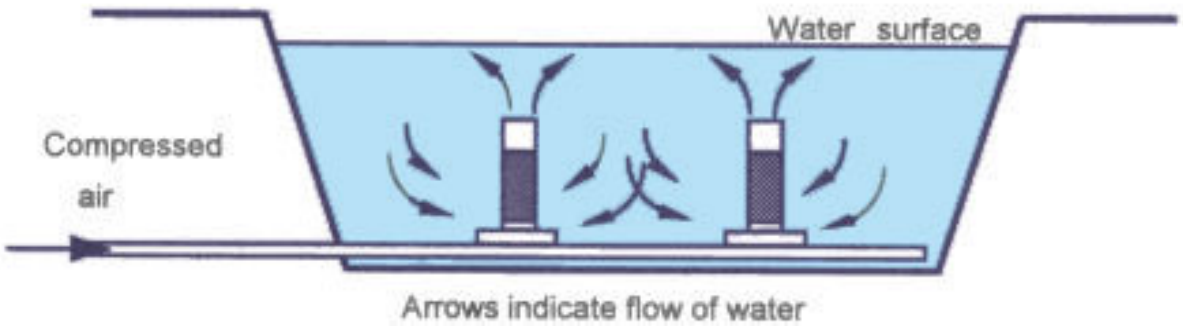


Fine and medium bubble diffusers



Coarse or Large Bubble Diffusers
With the small or fine bubble diffusers, it is important to use air free of particles that would otherwise clog them. Although somewhat less efficient for oxygen transfer, the coarse bubble diffusers are sometimes preferred because the presence of particles in the air is not a critical problem, and also for their lower cost and maintenance requirements. The diffusers are placed along air manifolds, close to the bottom of the aeration tanks.

The static aerators are vertical tubes placed at the bottom of the aeration tank, with packing material along its length. The compressed air is supplied from the bottom of the tubes, forcing a mixture of air and water through the packing, where most of the oxygen transfer to the wastewater takes place. They have been used mainly in aerated lagoons.



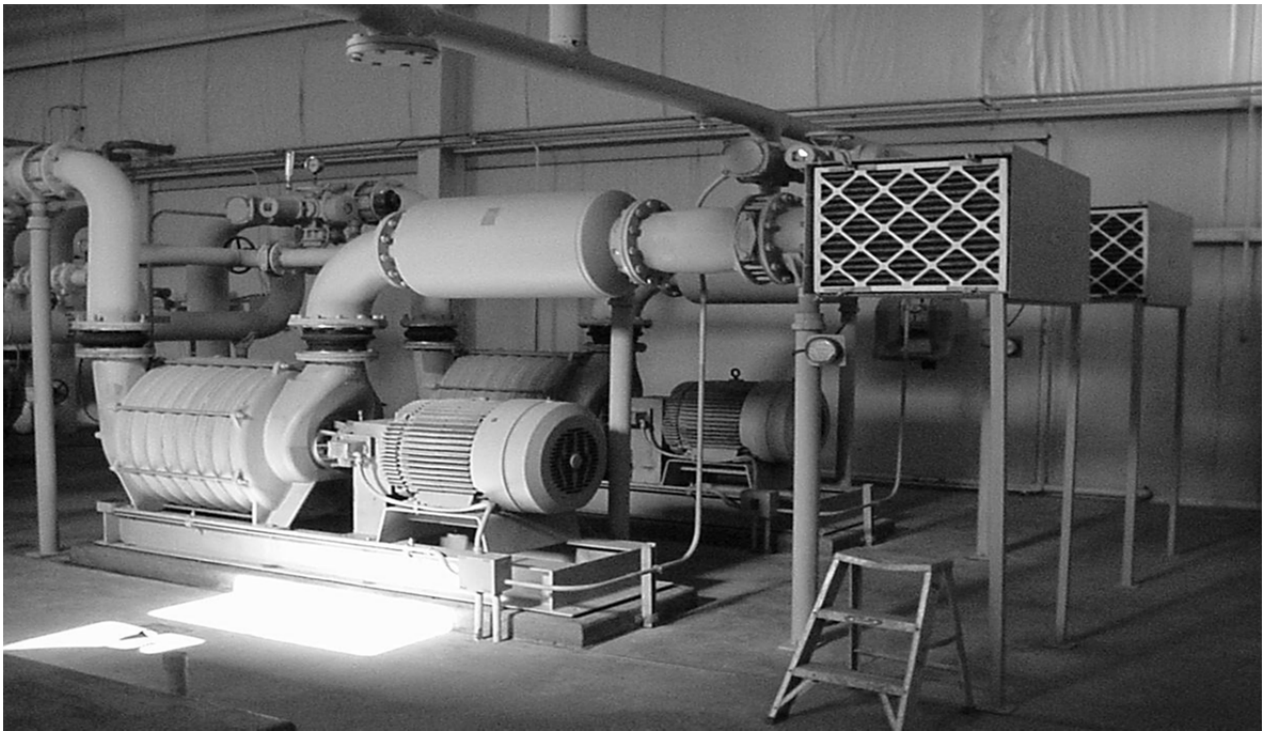
Oxidation ditches are used for nutrient removal.

Blowers

In the diffused aeration system, blowers are used to circulate the tank's contents by the air-lift effect. The air filter on the blower removes dirt from the air. Therefore, helps prevent diffuser clogging. Before all this begins, we need a power source to drive the blower. Usually, electric motors are used but in remote locations, gas or diesel engines can be used as well. In some states, solar energy is available to provide the power.

As illustrated in the photograph below, the rotation of the motor shaft is transferred to the blower shaft by means of a flexible coupling or through drive belts. The blowers that we will refer to are centrifugal blowers.

The centrifugal blower works like a centrifugal pump or a fan. Rotating impellers or fans cause movement of the air through the blowers. You have an intake side that takes in the air and the discharge side the forces the air out. The number of impellers you have will determine if it is a multi-stage or single-stage blower. The photograph below illustrates the major components of a centrifugal blower.



A lobe blower utilizes positive displacement; it also has an intake and a discharge side. The lobes turn in opposite directions in the casing. As they turn, the air is drawn in through the blower inlet and is trapped. The lobes keep turning, opening the blower discharge, and forcing the trapped air through the outlet. Usually, an electric motor drives the blower with belt pulleys or flexible couplings.

Before we continue let's review what you just read about the blowers and motors.

1. What are two ways that the motor and the blowers can be attached?

2. When using flexible couplings, what are some maintenance concerns to consider?

Blowers may be provided with additional equipment. For example, safeguards can be installed to protect equipment and operators. Temperature sensors can be used for bearing housing, vibration sensors protect the unit by shutting it down if limits are exceeded. Condensation drains should be provided on the bottom of blowers to drain off any accumulated moisture.

The compressed air from the blowers moves into a system of pipes and valves. The amount of air supplied from the blower is controlled by regulating valves mounted on the intake and/or discharge side of the blower. Usually butterfly valves are used and depending on your budget, you could have manually operated or use automation.

Blowers usually discharge to a common manifold, so check valves are installed at the discharge of each blower. The intake and discharge pipes are called the air mains. They are connected by a flexible connection to allow for vibration and heat expansion in the piping. In the winter months, the best place to be is in the blower room. There is a pressure relief valve on the discharge manifold to protect the blower from excessive back pressure overload. When this occurs the operator will be awakened on the midnight shift. Pressure gauges are used in several areas on the discharge side of the blowers. In some cases you may see them on the intake side for use in calculations of pump efficiency.

On the intake side, where air is supplied, you would have some type of filtering to remove dirt particles that could clog the diffusers. It also protects the blowers from excessive wear. Replaceable filter units are the simplest for operations. Bag house dust collectors are bulky and expensive, though maintenance may be less. In some cases, electrostatic precipitators may be an advantage, shocking if operators are not careful, in areas of poor air quality. Most systems have utilized pressure drop measuring to indicate when it is time to replace or clean the units.

The above photograph shows air being unevenly distributed.

Diffusers

There are many different design layouts and patterns of diffuser placement. Systems that allow longer and more complete contact between the air and the liquid are preferred. We will focus on fine bubble (porous) diffusers and coarse bubble (nonporous).

Coarse bubble diffusion devices, or large-hole diffusers, produce larger bubbles than porous plates, porous tubes, or synthetic socks. The larger bubbles provide less surface area for air-liquid contact and will result in less oxygen transfer efficiency than that obtained with fine bubble diffusers.

Answer this question:

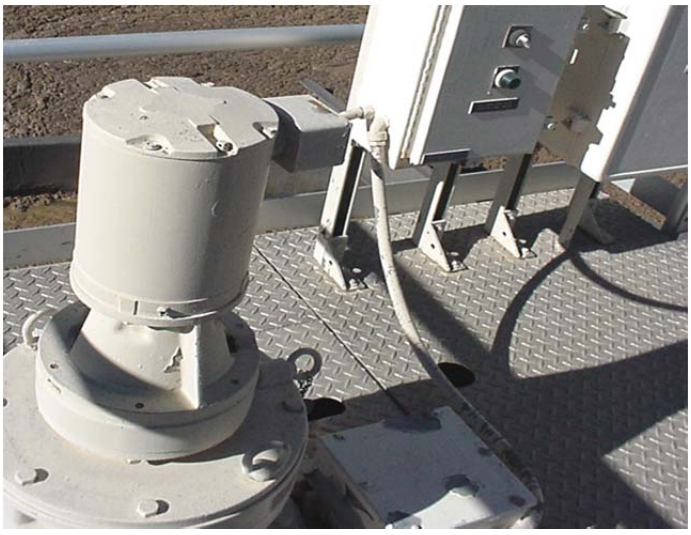
An air stone like those used in aquariums is a good example of a?

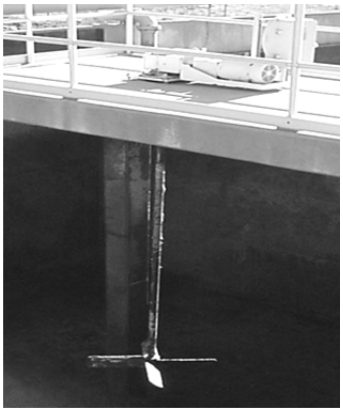
A. Porous material
B. Nonporous material

Mechanical Aeration

There are several main types of mechanical aeration devices. The floating and fixed bridge aerators are quite common. Some use a blade to agitate the tank's surface and disperse air bubbles into the aeration liquor. Others circulate the mixed liquor by an updraft or downdraft pump or turbine. This action produces surface and subsurface turbulence, while diffusing air through the mixed liquor.

The motor speeds are usually in the 1800 rpm range. This speed is reduced to the 30 to 70 rpm range with gear reducers.





Most vertical motors are mounted on a gear reduction unit as seen in the photograph on the right. The impeller drive shaft can be enclosed in a housing connected directly to the gear box. There is a bearing at the bottom of the shaft that steadies and aligns this shaft. This bearing needs lubrication, always check your manufactures recommendations.

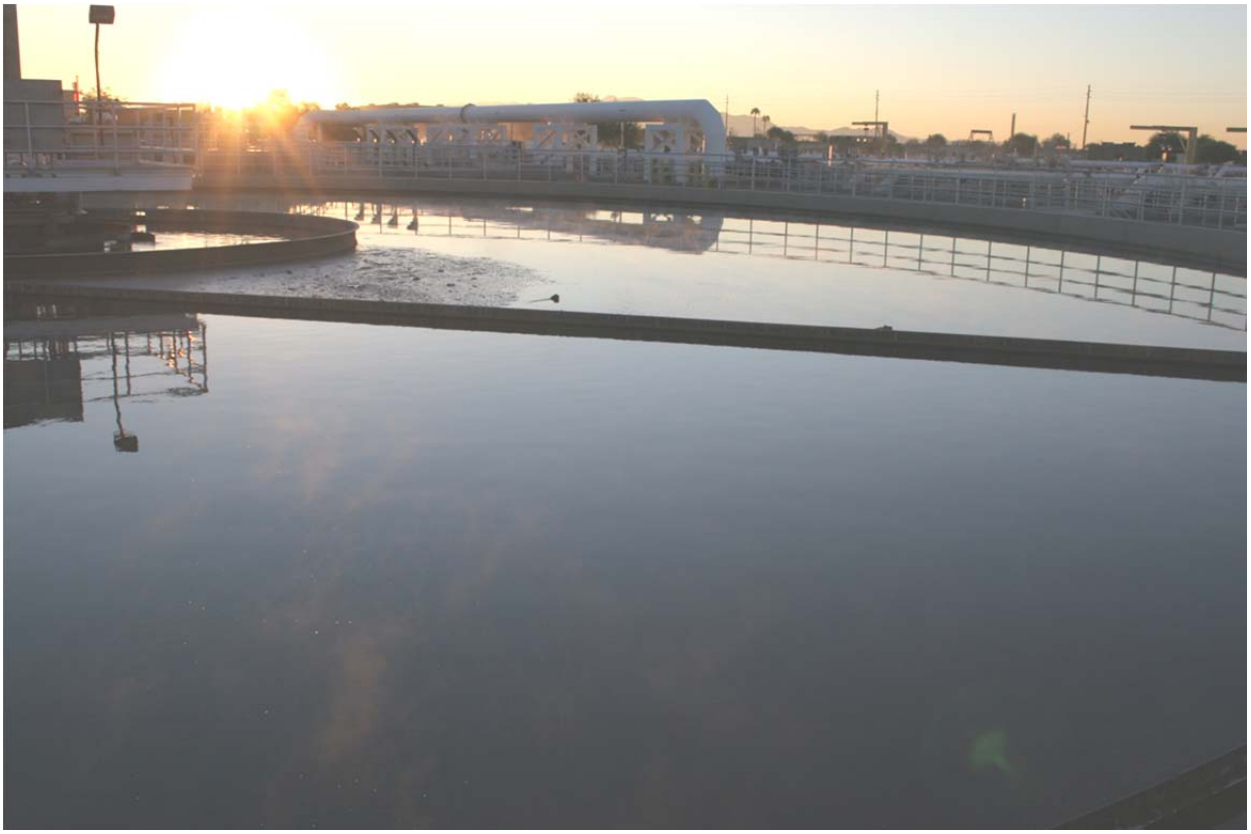
Some plants use an oxidation ditch in which rotating brushes, blades, or disks are rotated partially submerged in the mixed liquor. The turbulence produced traps the air bubbles and keeps the mixed liquor in motion.

Other systems use both compressed air and a mechanical device to trap the bubbles. In one such system, submerged turbine aeration, air is injected below a rotating turbine blade that shears and disperses the air.

Submerged turbine applications have also used a draft tube operating in a downdraft-pumping mode.

Jet and Aspirator

Aerators provide oxygen transfer by mixing pressurized air and water within a nozzle and then discharging the mixture into the aeration tank. The velocity of the discharged liquid and the rising air plume provide the necessary mixing action.



Secondary Clarifiers

Because microorganisms are continually produced, a way must be provided for wasting some of the generated biological solids produced. This is generally done from the round or rectangular shaped clarifiers.

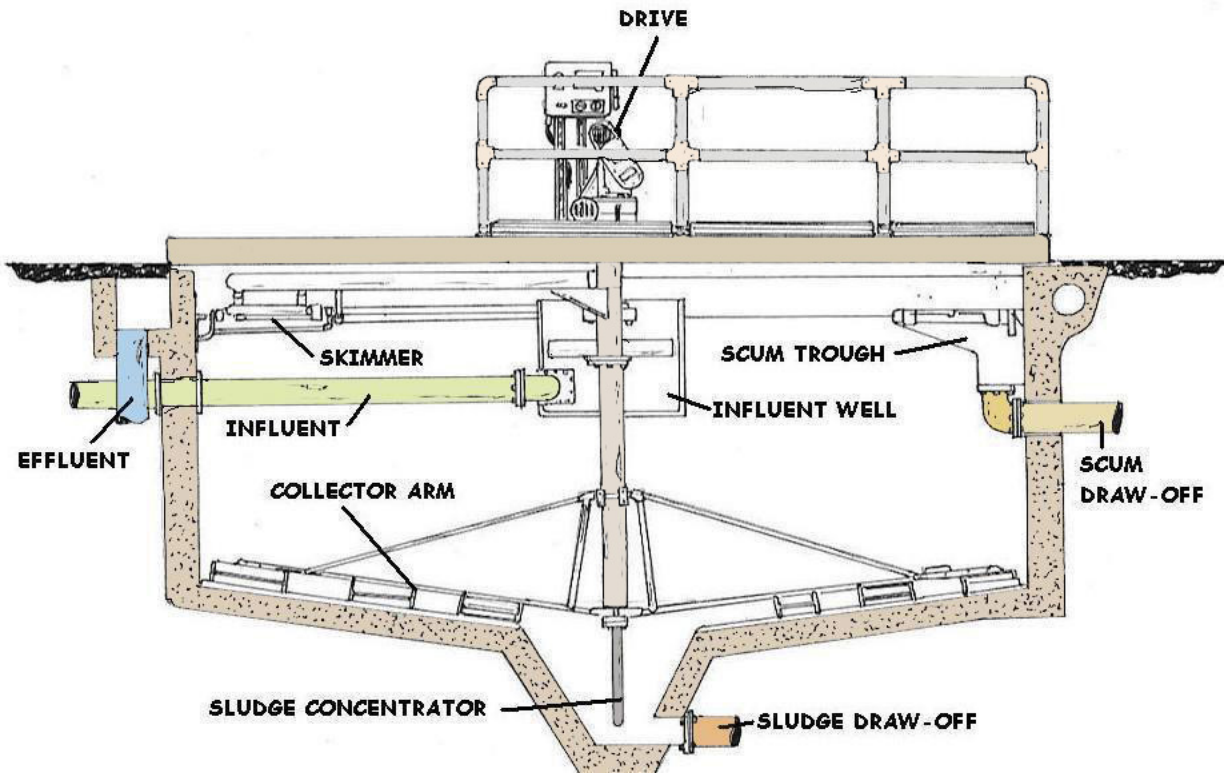
Let's first look at the components of a rectangular clarifier. Most are designed with scrapers on the bottom to move the settled activated sludge to one or more hoppers at the influent end of the tank. It could have a screw conveyor or a traveling bridge used to collect the sludge. The most common is a chain and flight collector. Most designs will have baffles to prevent short-circuiting and scum from entering the effluent.

The activated sludge is removed from the hopper(s) and returned by a sludge pump to the aeration tank or wasted. Since we mentioned return and waste what do the following terms represent?

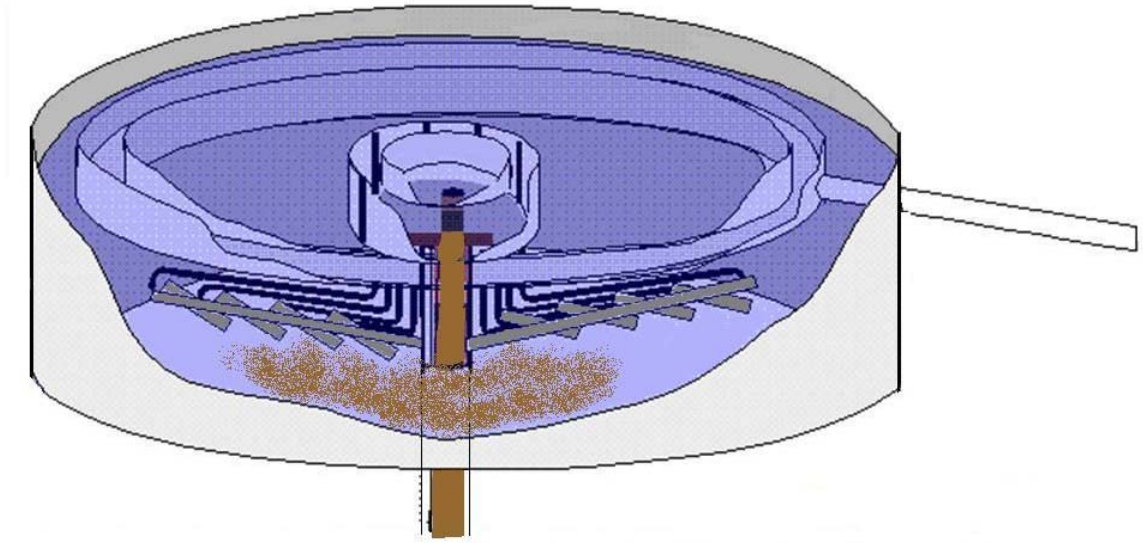
RAS:

WAS:

CLARIFIER



Clarifier - A settling tank used to remove suspended solids by gravity settling. Commonly referred to as sedimentation or settling basins, they are usually equipped with a motor driven chain and flight or rake mechanism to collect settled sludge and move it to a final removal point.

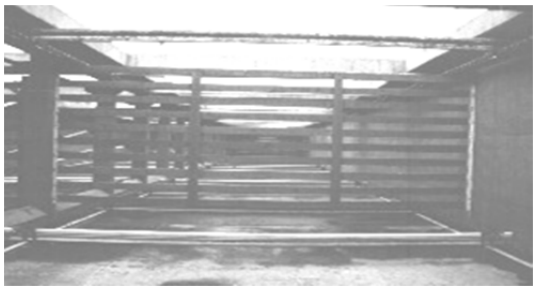


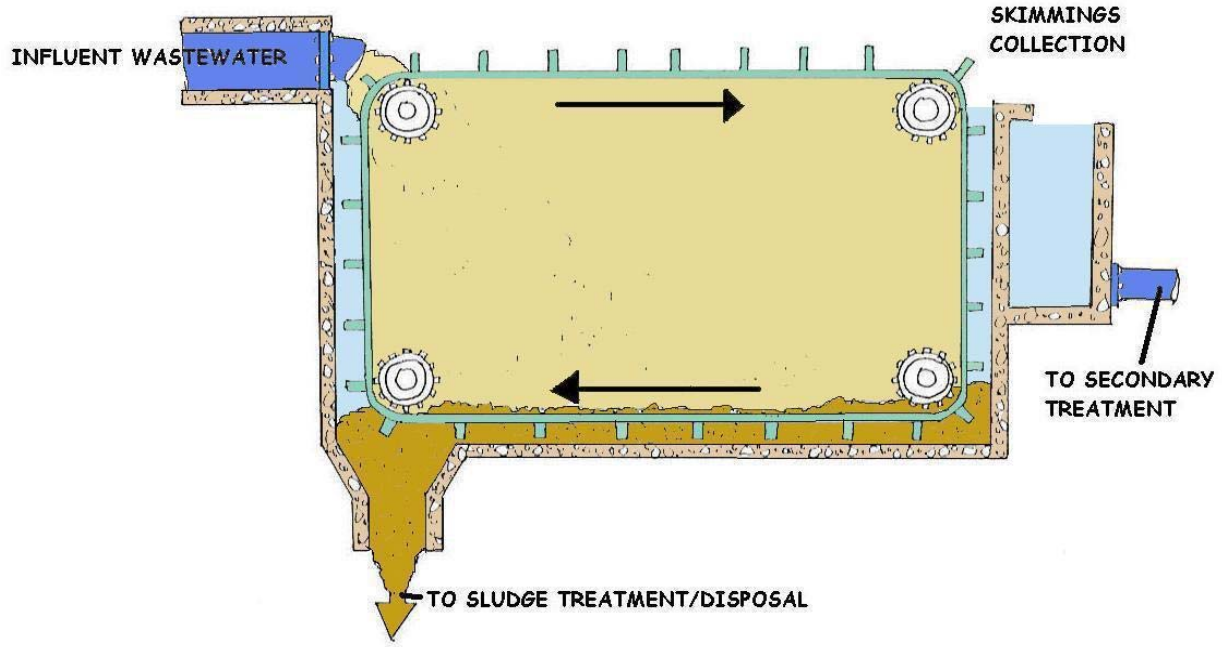
Scum Removal Equipment

Scum removal equipment is desirable on secondary clarifiers. Skimmers are either of the type that rotates automatically or manually. The most important thing to consider is the sludge and scum collection mechanism. We will talk about "*flights and chains*". They move the settled sludge to the hopper in the clarifier for return and they also remove the scum from the surface of the clarifier. The flights are usually wood or nonmetallic flights mounted on parallel chains. The motor shaft is connected through a gear reducer to a shaft which turns the drive chain. The drive chain turns the drive sprockets and the head shafts. The shafts can be located overhead or below.

Some clarifiers may not have scum removal equipment so the configuration of the shaft may vary. As the flights travel across the bottom of the clarifier, wearing shoes are used to protect the flights. The shoes are usually metal and travel across a metal track.

To prevent damage due to overloads, a shear pin is used. The shear pin holds the gear solidly on the shaft so that no slippage occurs. Remember, the gear moves the drive chain. If a heavy load is put on the sludge collector system, the shear pin should break. This means the gear would simply slide around the shaft and movement of the drive chain would stop.





Top photograph, a clarifier's raking mechanism. Bottom, scum armature equipment.

Scum Removal Equipment

In some circular or square tanks rotating scrapers are used. The diagram below shows an typical scum removal equipment.

The most common type has a center pier or column. The major mechanical parts of the clarifier are the drive unit; the sludge collector mechanism; and the scum removal system. There is also some related equipment that we will consider briefly. Let's look at the drive unit first. There are three main parts to the drive unit; the motor (or gear motor); the gear reducer; and the turntable.

The motor is connected to a gear reduction unit which is commonly connected to additional gearing. The drive cage is rotated around a center column by the motor and gear reduction unit. Although the drive motor runs at about 1800 rpm, the gear reducer lowers the output speed so that the sludge collector mechanism goes through one revolution every 20 to 30 minutes. Usually, the motors used on clarifier mechanisms are totally enclosed, fan cooled motors, suitable for outside operation.

The horsepower of the motor is dependent on the size of the clarifier. The motor drives the chain and sprocket which drives the worm gear. The worm gear drives the gear that is mounted on a shaft that drives the turntable. The motor shaft speed is reduced by a series of gear reducers.

We looked at the main parts of the drive unit. Now let's take a look at the sludge collector and the scum removal system mechanism. The main parts of the unit are: the rake arm; the scraper blades; the adjustable squeegees; the surface skimmer; the scum baffles; and the scum box.

The surface skimmer rotates at the same speed as the collector mechanism and is usually supported by the collector rake arm. The scum baffle prevents scum from flowing over the effluent weir. The surface skimmer collects the scum and deposits it in the scum box. The stilling well or influent baffle projects above the liquid and directs the influent downwards to assist in settling suspected solids and reduce short circuiting. Another important part of the secondary clarifier is the effluent weir, launder and pipe. An effluent weir goes around the circumference of the tank and allows clarified liquid to flow evenly from the tank. The effluent launder collects the tank overflow and takes it to a low point in the launder where a pipe is used to take the effluent to the chlorine contact basin or other means of treatment.

Some clarifiers may have a scum trough heater. The scum removal system rotates around the clarifier at a very slow rate. In subfreezing temperatures, the scum box and pipe could freeze. This problem can be overcome by using immersion heaters, or putting infrared lamps over the scum box. Some clarifiers are covered.

As you have previously read, depending on the design and operation of the process, activated sludge has several interrelated components:

1. Single aeration tank or multiple aeration tanks designed for completely mixed or plug flow.

2. An aeration source to provide adequate oxygen and mixing: sources can be compressed air, mechanical aeration, or pure oxygen.

3. A clarifier to separate the biological solids (activated sludge) from the treated wastewater.

4. A means of collecting the biological solids in the clarifier and recycling most of them (return activated sludge, RAS) to the aeration tank.

5. A means of removing or wasting excess biological solids (waste activated sludge, WAS) from the system.

Review Process Goals

As previously noted, the activated sludge process can be used to remove carbonaceous BOD and also ammonia (nitrification). We can take the wastewater oxygen demand separated into two categories: carbonaceous and nitrogenous.

Carbonaceous BOD Removal
The carbonaceous demand should be expressed as a function of the number of days that the demand will be measured; 3-day, 5-day (most common), 7-day, and 20-day time periods are commonly used. To obtain only carbonaceous oxygen demand, it may be necessary to inhibit nitrification by adding chemicals.

The rate and extent of BOD5 (5-day BOD) removal in primary treated (settled) or untreated wastewater depends on the relative quantities of soluble, colloidal, and suspended BOD5, and a soluble BOD5 content of approximately 20 to 40% of the total. These proportions may vary, particularly in warmer climates where long collection system residence times and the higher wastewater temperatures may result in a higher proportion of soluble BOD5. This is caused by the bacterial degradation of a portion of the colloidal and settleable fractions.

With typical municipal wastewater, a well-designed activated sludge process should achieve a carbonaceous, soluble BOD5 effluent quality of 5mg/L or less. Similarly, with clarifiers designed to maximize solids removal at peak flows and adequate process control, the average SS in the effluent should not exceed 15 mg/L. On a practical basis, an effluent with 20/20 mg/L BOD5 and SS should be attained, assuming proper operation. Potential capabilities of the process are 10/15 mg/L Bod5 and SS. To consistently achieve values lower than 10/15 mg/L, some type of tertiary treatment is required.

Nitrification
Of the total oxygen demand exerted by the wastewater, there is often a sizeable fraction associated with the oxidation of ammonia to nitrate. The autotrophic bacteria Nitrosomonas and Nitrobacter are responsible for this two-state conversion. Being autotrophic, these nitrifying organisms must reduce oxidized carbon compounds in the wastewater, such as CO_2 and its related ionic species, for cell growth. As a result, this characteristic markedly affects the ability of the nitrifying organisms to compete in a mixed culture.

The nitrifying bacteria obtain their energy by oxidizing ammonia nitrogen to nitrite nitrogen and then to nitrate nitrogen. Because very little energy is obtained from these oxidation reactions, and because energy is needed to change CO_2 to cellular carbon, the population of nitrifiers in activated sludge is relatively small. When compared to the normal bacteria in activated sludge, the nitrifying bacteria have a slower reproduction rate.

Nitrifying organisms are present to some extent in all domestic wastewaters. However, some wastewaters are not nitrified in existing plants because they are designed for the higher growth rate of bacteria responsible for carbonaceous removal. As the MCRT is increased, nitrification generally takes place. The longer MCRT prevents nitrifying organisms from being lost from the system when carbonaceous wasting occurs or, more accurately, the longer MCRT permits the build-up of an adequate population of nitrifiers.

Because of the longer MCRT required for nitrification, some systems are designed to achieve nitrification in the second stage of a two-stage activated sludge system.

The oxygen demand for complete nitrification is high. For most domestic wastewaters, it will increase the oxygen supply and power requirements by 30 to 40% because complete nitrification requires from 4.3 to 4.6 lb. of oxygen for each lb. of ammonia nitrogen (4.3 to 4.6 mg/mg) converted into nitrate, and wastewaters generally contain 10 to 30 mg/L of reduced nitrogen. Nitrification systems generally are not operated at intermediate (40 to 80%) removals; stable operation is achieved when essentially complete nitrification (greater than 90%) occurs.

Minimum acceptable dissolved oxygen (**DO**) concentrations of 2 to 3 mg/L have been reported, but nitrification appears to be inhibited when the oxygen concentration is lower than 1 mg/L.

Optimum growth of nitrifying bacteria has been observed in the pH range of 8 to 9 although other ranges have been reported. A substantial reduction in nitrification activity usually occurs at pH levels below 7, although nitrification can occur at low pH.

While nitrification occurs over a wide temperature range, temperature reduction results in a slower reaction rate.

The temperature effect is made less severe by increasing the MCRT. During the conversion of ammonia to nitrate, mineral acidity is produced. If insufficient alkalinity is present, the system's pH will drop and nitrification may be inhibited.

Bacteria Highlights
A change in the numbers or predominance of microorganisms in activated sludge is usually gradual. The time required for a complete shift from one species to another will normally be seen in: 2 to 3 MCRT's.

A large amount of long filamentous bacteria will prevent good settling. The liquid above this mass is called the supernatant.

Endogenous respiration of microorganisms in an extended aeration plant will complete the oxidation process of an organic material.

The bug Nocardia causes frothing.

Saprophytic type bacteria produces the most acid in an anaerobic digester.

The best location for microscopic examination of activated sludge in a conventional system is at the effluent end of the aeration system. The examination can reveal a predominant number of rotifers and nematodes, this condition may indicate that the F/M ratio is too low and this would be normal in an extended aeration process.

Food to microorganism ratio. A measure of food provided to bacteria in an aeration tank.

$$\frac{\text{Food}}{\text{Microorganism}} = \frac{\text{BOD, lbs/Day}}{\text{MLVSS, lbs}}$$

$$= \frac{\text{Flow, MGD} \times \text{BOD, mg/}L \times 8.34 \text{ lbs/gal}}{\text{Volume, MG} \times \text{MLVSS, mg/}L \times 8.34 \text{ lbs/gal}}$$

$$\text{or} \quad = \frac{\text{BOD, kg/day}}{\text{MLVSS, kg}}$$

Separate Stage Nitrification and Denitrification Systems

Suspended Growth Nitrification

Single-sludge systems for BOD removal and nitrification require that the biomass inventory be retained long enough to establish a stable population of nitrifiers and that the HRT be such that the biomass can react with the ammonia-nitrogen entering the system. The overall approach for designing such systems is to determine the target SRT for the system based on influent characteristics (i.e., BOD, ammonia-nitrogen, organic nitrogen), environmental conditions such as temperature and flow characteristics (i.e., average daily, maximum monthly, diurnal peak).

Most activated sludge treatment plants will readily nitrify if they have sufficient aerobic SRT and can deliver sufficient oxygen maintaining 2 mg/L DO or greater. For plants having difficulty in nitrifying due to insufficient tank volume, there are some emerging technologies which can improve the process.

One of these is bioaugmentation. Bioaugmentation is accomplished by seeding the activated sludge process with an external source of nitrifying bacteria (also known as external bioaugmen-tation) or making process improvements to increase the activity of or enrich the nitrifier population (also known as *in situ* bioaugmentation).

External bioaugmentation uses either commercial sources of nitrifiers or sidestream processes to grow nitrifiers onsite. Early experiences with commercial sources were not consistent, so most work to date has been with sidestream production onsite (USEPA, 2008a). Two patented sidestream configurations for external bioaugmentation are the Single reactor High-activity Ammonia Removal Over Nitrite (SHARON) process and the In-Nitri® process. Both provide high temperature sidestream nitrification using ammonia from the anaerobically digested sludge dewatering liquid or digested supernatant. The nitrifiers grown in the sidestream reactor are fed to the main liquid treatment stream.

Both use flow through reactors with hydraulic retention times (HRT) in the 2 to 3 day range. In the SHARON process, nitrification is stopped mainly at nitrite by such process control methods as low DO concentration, low pH and/or low SRT. Full-scale operating systems for the SHARON process include installations at Utrecht, Rotterdam, Zwolle, Beverwijk, Groningen, The Hague in the Netherlands, and a system in New York City. Seeding from a diffused air biological nutrient removal process to stimulate nitrification in a parallel oxygen process has proved successful at a number of locations (Bott et al., 2007). Emerging *in situ* bioaugmentation technologies used to enhance nitrifier growth and shown to be successful in bench, pilot, and/or full-scale trials are described briefly below (USEPA, 2008a):

• The Bio-Augmentation Regeneration/Reaeration (BAR) process was developed in the U.S. and is identical to the Regeneration-DeNitrification (R-DN) process developed independently in the Czech Republic. It works by recycling ammonia-laden filtrate or centrate from dewatering of aerobically digested sludge to the head of the aeration tank. The sidestream is fully nitrified, seeding the aeration tank with additional nitrifying bacteria which allows for reduced SRT.

• Aeration Tank 3 (AT3) is similar to the BAR process except that it sends a smaller fraction of the return activated sludge (RAS) to the aeration tank in order to stop the nitrification process at the nitrite stage.

• Bio-Augmentation Batch Enhanced (BABE) process uses a SBR to grow nitrifiers by feeding it RAS and reject water from the sludge dewatering process. After treatment, concentrated nitrifiers are recycled to the head of the aeration tank.

• The Mainstream Autotrophic Recycle Enhanced N-removal (MAUREEN) Process was developed for the two-sludge treatment configuration at the Blue Plains Advanced Wastewater Treatment Plant in Washington, DC. The process involves sidestream treatment of WAS from the second stage to preferentially select AOB for bioaugmentation to the first sludge stage.

Attached Growth Nitrification

Attached growth processes will also nitrify. Trickling filters and rotating biological contactors (RBCs) have historically been used for biological treatment of wastewater and can achieve nitrification with a low organic loading and a relatively high media volume. Typically, nitrification is achieved on the media after most of the BOD is removed since the heterotrophic population competes with the nitrifying organisms for oxygen and space on the media.

A major disadvantage of these technologies compared to suspended growth systems is that denitrification is fully dependent on addition of a supplemental carbon source. Suspended growth processes, on the other hand, can be designed to denitrify 80 percent or more of nitrate using the incoming BOD as the carbon source, which is a lower cost solution.

Consequently, trickling filters and RBCs have fallen out of favor for nutrient removal applications. In recent years, manufacturers have developed new technologies called biological aerated filters (BAF) to achieve BOD removal and nitrification. USEPA (2008a) identifies two existing BAF designs as established technologies: the Biofor® system and the Biostyr® system. The Biofor® filtration system is a fixed bed, upflow system with a dense granular media that is designed to expand during filtration. Air is sprayed into the filter to maintain an aerobic environment. The Biostyr® system is similar but uses a media that is less dense than water and held in place during operation by a screen at the top of the cell.

BAF can be configured in series to remove BOD in one unit and ammonia-nitrogen in the next or it can be designed for BOD removal and nitrification in a single unit depending on process goals. Advantages of BAF include its smaller footprint, higher hydraulic loading rates, and less susceptibility to washout than suspended sludge systems (Verma et al., 2006).

Another fixed film process that has gained popularity lately is moving bed biofilm reactors (MBBR). These reactors involve biofilm attached to a plastic media in a series of fluidized bed reactors. The plastic media help promote specialization of the biofilm within each reactor for either nitrification or denitrification (WEF and ASCE, 2006). Mixers or medium bubble diffuse aeration are used to keep the media suspended, depending on whether the system is anaerobic or aerobic. MBBR has a shorter SRT and smaller footprint than activated sludge processes. It has also proven to be effective in cold temperatures (Bott et al., 2007).

Separate-Stage Denitrification

A separate-stage denitrification system may be appropriate for plants that are regularly achieving nitrification and need to add denitrification capabilities. Attached growth systems (denitrifying filters) are more common than suspended growth systems, although suspended growth systems have been used for some treatment plants. Suspended growth reactors typically have short SRTs (2 to 3 hrs.) and a small aerated zone following the denitrification zone to oxidize excess methanol and release contained nitrogen gas bubbles (WEF and ASCE, 2006).

Denitrification filters typically have a small footprint compared to suspended growth systems and have the added advantage of achieving denitrification and solids removal simultaneously. They were first installed in the 1970s and have evolved into two main process configurations (USEPA, 2007c):

• Downflow denitrification filters are deep bed filters consisting of media, support gravel, and a block underdrain system. Wastewater flow is directed over weirs onto the top of the filter where a supplemental carbon source, typically methanol, is added. Backwashing (typically air scouring and backwashing with air and water) is conducted at regular intervals to remove entrapped solids from the filter.

During operation, nitrate is converted to nitrogen gas and becomes entrained in the filter media, increasing head loss through the filter. To release entrained nitrogen, most denitrification systems have a nitrogen-release cycle operation that essentially “bumps” the filter by turning on the backwash pump(s) for a short period of time.

• Upflow continuous backflow filters do not have to be taken off-line for backwashing, as it is an integral part of the filtering process. Wastewater enters the bottom of the filter where a carbon source, typically methanol, is added. Water flows up through an influent pipe and is dispersed into the filter media through distributors. Filtered water discharges at the top of the filter. Filter media continuously travels downward, is drawn into an airlift pipe at the center of the filter, and is scoured before being returned to the filter bed.

Performance of denitrifying filters depends on many factors including:
• Influent weir configuration
• Filter media
• Underdrain system
• Backwash system
• Flow and methanol feed control

One wastewater system in Connecticut reported that key design issues for them were influent piping design to minimize aeration, maintaining a consistent flow to the filters, and control of methanol feed based on influent COD (Pearson et al., 2008).

The tubes held in this photograph should be placed in an autoclave. One tube is a standard or QA and the other would indicate contamination.

The refrigerated automatic WWT sampler will have a Data programmer that will allow you to set the time to collect the sample or samples. This machine can also measure the amount of the sample. These can also be used for the collection of composite samples. Sometimes you will see a pH probe with real-time readings sent to the Operator's Command Center. These are a common sight at most wastewater plants and SIUs.

Key Design and Operational Issues

Temperature
In general, as temperature of the wastewater increases, the rate of nitrification and denitrification increases. For the typical range of liquid temperatures between 10 and 25^{o} C, the nitrification rate will approximately double for every 8 to 10^{o} C increase in temperature (WEF and ASCE, 2006). Rapid decreases in temperature without acclimation time will, however, cause even slower nitrification rates than predicted, strictly by the temperature change. Denitrification rates will also increase with increasing temperature, although not at the same magnitude as nitrification rates.

Dissolved Oxygen
Nitrifying bacteria are also more sensitive to DO levels as compared to aerobic heterotrophic bacteria, with growth rates starting to decline below 3 to 4 mg/L with significant reduction below 2
mg/L. The nitrification rate at a DO concentration of 0.50 mg/L is only about 60 percent of that at a 2.0 mg/L DO concentration. Studies have shown that the amount of oxygen available to nitrifying bacteria can be limited by floc size, requiring higher bulk DO concentrations under higher organic loading conditions (Stenstrom and Song, 1991). At DO concentrations less than 0.5 mg/L, the effect is greater for *Nitrobacter* than for *Nitrosomonas*. This can result in higher NO2-N in the effluent and have a negative impact on chlorine disinfection as 1 g of NO2-N consumes 5 g chlorine for oxidation. DO must normally be less than 0.2 to 0.5 mg/L, otherwise there will be inhibition of the denitrification process.

pH and Alkalinity
Nitrification generally operates well within a pH range of 6.8 to 8.0 (WEF and ASCE, 2006). At lower pH values the nitrification rate is much slower and at pH values near 6.0 the nitrification rate may only be about 20 percent of that with a pH of 7.0 (Tchobanoglous et al., 2003). Alkalinity is consumed during the nitrification process but partially replenished (up to 62.5 percent) during the denitrification process. Depending on the influent wastewater alkalinity, there may be a sufficient alkalinity reduction due to nitrification to decrease to unacceptable levels. Addition of chemicals such as lime, sodium hydroxide, or soda ash can be used to replace the alkalinity consumed by nitrification to maintain acceptable pH levels.

Carbon Sources for Denitrification
Denitrifying bacteria need a readily available carbon food source, such as soluble BOD, to ultimately convert nitrate to nitrogen gas. WWTPs that meet very low total nitrogen limits typically use a secondary anoxic zone in which supplemental carbon is added. Supplemental sources can be “internal” such as fermented wastewater or sludge, or “external” sources such as purchased chemicals.

Methanol is currently the most common external carbon source used in denitrification because of it low cost. It has several drawbacks, however, namely:
• It is highly flammable and implicated in some storage tank explosions and fires (Dolan, 2007); however with proper design and operation problems can be minimized.
• It is not the most efficient source for most treatment configurations.
• Costs have begun to fluctuate widely (deBarbadillo et al., 2008).
• Availability is a problem in some areas (Neethling et al. 2008).
• Reported low growth rates under cold temperatures (Dold et al. 2008).

Other sources of carbon include ethanol, acetic acid, corn syrup, molasses, glucose, glycerol, and industrial waste products. The WEF Nutrient Challenge Research Plan (2007) identified research on alternative carbon sources as priority for operators, owners, and engineers of wastewater systems. In December of 2007, the 2nd External Carbon Workshop was held in Washington, DC to discuss the state of the technology and research needs. WERF is also currently formulating a standard protocol for evaluation of external carbon alternatives.

Nitrification Inhibition from Toxic Chemicals
Nitrifying bacteria are very sensitive to heavy metals and other inorganic compounds in waste-water. The Local Limits Development Guidance Manual (USEPA 2004) has been the main source of information on inhibitory effects for a variety of wastewater treatment processes including nitrification. Appendix G of the 2004 version provides a summary table with the reported range of nitrification inhibition threshold levels for a number of metals, non-metal inorganics, and organic compounds. Actual inhibitory effects are site-specific and depend on many factors including the nature of biodegradable organic material, microorganism speciation, acclimation effects, temperature, and water quality conditions.

Wet Weather Events
Wet weather events can increase inflow and infiltration into the collection system and subsequently increase the hydraulic load to the wastewater treatment plant. This can in turn reduce the SRT leading to reduced performance of nitrification process units. In addition, wet weather flows have different characteristics than typical wastewater influent flow and can be less favorable for nitrification and denitrification. Conditions that are less favorable for nitrification include decreased alkalinity and sudden temperature drops. Lower biodegradable COD concentrations and increased DO make wet weather flows less amenable to denitrification.

Flow equalization basins can be used to handle wet weather events; however, this requires available space and capital investment. USEPA (2008a) identifies a number of innovative storage and treatment technologies used to manage influent flows during wet weather events.

Guidance for Selecting Process Modifications
Nitrogen removal requires first that a biological nitrification process be present or that the facility be modified to accomplish nitrification. Considerably more volume is needed for activated sludge nitrification compared to designs for BOD removal only. If there is insufficient space to accommodate the increased volume, suspended growth or hybrid process options that require less space such as the MBR process or IFAS systems with suspended media in the activated sludge process should be considered. Another option is to use a fixed film nitrification process after the suspended growth process clarification step. This could be a BAF or a plastic media trickling filter. However, if nitrogen removal is required, an exogenous carbon source is needed, which has higher operating costs than using the influent BOD for denitrification.

Nitrification systems need sufficient oxygen transfer for ammonia oxidation in addition to BOD removal. Such systems should consider the impact to diurnal loadings and ammonia addition in recycle streams. The influent TN concentration may have daily peak values that are 1.5 to 2.0 times the daily average loading. Higher peak loadings require longer SRTs to assure that sufficient nitrifying bacteria are present to remove ammonia at a greater rate, while maintaining a low effluent ammonia concentration. Often anaerobic digester sludge dewatering operations occur during the day and produce return recycle streams high in ammonia concentration (500-1000 mg/L) at times that coincide with the high influent diurnal ammonia loads. Recycle equalization or treatment helps to provide a more stable nitrification system and lower effluent NH3-N concentrations.

In many cases, it is advantageous to incorporate a denitrification pre-anoxic step with nitrification (MLE process) due to the many benefits and improved operational stability. The advantages include
1) less aeration energy as the nitrate produced can be used for BOD removal,
2) the production of alkalinity to offset the alkalinity used by nitrification, which in some cases eliminates the need to purchase alkalinity, and
3) a more stable, better settling activated sludge process as the anoxic-aerobic processes favor good settling floc-forming bacteria over filamentous growth.

The effluent nitrogen goals greatly affect the process design choices and system operation. For an effluent goal of 10 mg/L TN, an MLE process is often sufficient for activated sludge treatment with secondary clarifiers or membrane separation. However, with water conservation leading to more concentrated wastewater, these processes alone may not be sufficient due to the fact that they are limited to 80-85% removal of the influent TN.

For TN effluent goals of 3 to 5 mg/L or lower, some form of post anoxic treatment is generally needed. One option is to convert an MLE process to a Bardenpho process by adding another anoxic aerobic set of tanks. Although the endogenous respiration rate of the bacteria can be used to consume nitrate in the post anoxic tanks, it is often necessary to add an exogenous carbon source. Other alternatives to using exogenous carbon sources include denitrification filters instead of adding more activated sludge tank volume, step feed with carbon addition in the last pass, and IFAS processes.

Denitrification processes require sufficient carbon to drive the nitrate/nitrite reduction reactions. Characterization of the influent wastewater with regard to its organic strength and soluble fraction and the TN and ammonia concentrations is needed to fully understand a system's carbon needs. In addition, design and operating methods that eliminate or minimize DO feeding to anoxic zones can reduce the amount of exogenous carbon needed and provide a more stable operation. Low DO zones prior to downstream anoxic tanks or for withdrawal of recycle to preanoxic zones should be considered.

Impacts on Sludge Production and Handling

It has been documented by both research and full scale experiments that BOD removal by activated sludge using nitrate as the electron acceptor instead of DO will result in a 20% or more reduction in waste activated sludge (WAS) production for the same operating conditions. Full-scale investigations near Melbourne, Australia achieved as high as a 40% reduction in WAS, and implementation of nitrogen removal at the York River, VA, plant resulted in a reduction of more than 50% in WAS production. The impact this will have on total sludge production by a treatment plant will depend upon how much waste sludge is produced by other treatment units such as primary clarifiers and chemical treatment with precipitating chemicals.

Additionally, implementation of nitrogen removal at conventional activated sludge plants can improve the thickening characteristics due to decreasing the amounts of filamentous bacteria in the activated sludge. If an external carbon source is added to improve the rate of denitrification, there will be an increase in WAS production compared to when no external carbon source is added. If an external carbon source is used to supplement denitrification, it is likely that the small increase in solids production will be offset by endogenous respiration due to longer SRTs. Solids produced from nitrogen removal processes generally thicken and dewater well and show no negative impact on any solids processing system.

What is in Municipal Wastewater?

Municipal wastewater consists primarily of domestic wastes from households and industrial wastewater from manufacturing and commercial activities. Both types of wastewater are collected in sanitary sewers, and are usually treated at a municipal wastewater treatment plant. After treatment, the wastewater is discharged to its *receiving water (i.e.,* a river, an estuary, or an ocean).

Wastewater entering a treatment plant may contain organic pollutants (including raw sewage), metals, nutrients, sediment, bacteria, and viruses.

Toxic substances used in the home – motor oil, paint, household cleaners, and pesticides - or substances released by industries, also make their way into sanitary sewers.

Industrial processes, such as steel or chemical manufacturing, produce billions of gallons of wastewater daily. Some industrial pollutants are similar to those in municipal sewage, but often are more concentrated. Other industrial pollutants are more exotic and include a variety of heavy metals and synthetic organic compounds. In sufficient dosages, they may present serious hazards to human health and aquatic organisms. Unlike municipal or industrial sources of pollution, which come from a single discrete facility, other sources are usually more diffuse. For example, rainwater or snowmelt washing over farmlands may carry topsoil and fertilizer residues into nearby streams.

Stormwater
This type of runoff, called *stormwater,* may carry oil and gasoline, agricultural chemicals, nutrients, heavy metals, and other toxic substances, as well as bacteria, viruses, and oxygen-demanding compounds. A recent EPA study indicated roughly one-third of identified cases of water quality impairment nationwide are attributable to stormwater, whether from farmland, streets, parking lots, construction sites, or other sources.

Animal Feeding Operations (**AFOS**)
Animal Feeding Operations are livestock-raising operations, such as hog, cattle and poultry farms, that confine and concentrate animal populations and their waste. Animal waste, if not managed properly, can run off to nearby water bodies and cause serious water pollution and public health risks. There are approximately 450,000 AFOs in the United States.

Acid Mine Drainage
Acid Mine Drainage is one of the most significant environmental impacts resulting from past and current mining activities. It has been cited as a major cause of stream pollution in northern Appalachia (PA, W. VA, VA, MD); more than 50 percent of stream miles in PA and WV do not meet water quality standards because of acid mine drainage impacts. In addition, there are an estimated 200,000 abandoned hardrock mines nationwide and somewhere between 2,000 and 10,000 active ones. Some of these mining operations produce waste material and other conditions that result in acid mine drainage as well as discharges of heavy metals which affect aquatic life and drinking water sources.

Biological Phosphorus Removal and Combination Processes

This section provides an overview of the principles behind biological phosphorus removal (BPR). It describes existing configurations that can achieve phosphorus removal and in many cases, simultaneous nitrogen removal. Key operational issues, impacts on sludge handling, and a summary of ongoing research related to BPR removal are also provided.

Principles

Biological phosphorus removal is achieved by contacting phosphorus accumulating organisms (PAOs) in the RAS with feed, containing volatile fatty acids (VFA), in a zone free of nitrates and DO (anaerobic zone). Phosphorus is released in this zone providing energy for uptake of VFAs that are polymerized and stored inside the PAO cells. The anaerobic zone is followed by an aeration zone where the polymerized VFAs are metabolized and phosphorus is taken up again to store excess energy from the metabolism.

The phosphorus content of the mixed liquor suspended solids (MLSS) would be similar to that of the waste activated sludge (WAS). When nitrification occurs in the aeration basin, nitrates will be present in the RAS, resulting in some metabolism of the VFA before storage, thereby reducing subsequent phosphorus uptake. Some form of denitrification (anoxic zones) must be used to reduce/remove the nitrates from the RAS. The process flow sheets now known as Pho-redox (A/O) and 3 Stage Pho-redox (A2/O) as well as the modified Bardenpho process were first published by Barnard (1975) as the Pho-redox flow sheets for the removal of phosphorus. The theory for the functioning of the PAO was first suggested by Fuhs & Chen (1975).

Fuhs & Chen Theory

PAOs have the ability to store a large mass of phosphorus in their cells in the form of polypho-sphates. Polyphosphates are formed by a series of high-energy bonds. The organisms can subsequently get energy from breaking these bonds. The polyphosphate globules within the cells
function just like energy storage batteries. The storage of polyphosphates (energy), takes place in the aeration zone. In the anaerobic zone, these obligate aerobic bacteria can take up short chain VFA such as acetate and propionate and store them in the form of intermediate products such as poly-β-hydroxybutyrate (PHB). The energy for transferring the food across the cell membranes in the anaerobic zone is derived from breaking phosphorus bonds. Excess phosphates are released to the liquid in the anaerobic zone.

Some magnesium and potassium ions are co-transported across the cell walls with phosphates. PAOs can only get energy from the food they have taken up in the anaerobic zone when they pass to the aerobic zone where oxygen is available. They use oxygen to metabolize the stored products, deriving enough energy to take up all the released phosphates as well as those in the influent, and store them in the cells. The WAS will contain sufficient phosphate-enriched PAOs to remove most of the phosphorus from the waste steam once enhanced BPR is established.

The right carbon source, in this case a combination of acetates and propionates, is essential for
BPR. The wastewater characteristics are thus important. In general, it can be said that you need at least 40:1 COD:TP or about 18:1 BOD5:TP in the process influent wastewater to reduce effluent phosphorus to less than 1.0 mg/L. In addition, some of the COD should consist of short chain VFAs. More COD may be required if nitrates must also be denitrified.

Biological phosphorus removal can work in with or without nitrification. When nitrification occurs, denitrification within the process is important to reduce the nitrates that may be returned with the RAS. While the anaerobic zone serves mostly as a contact zone for VFAs and PAOs, some fermentation of easily biodegradable carbon compounds (rbCOD) to acetate and propionate may take place. In most plants the readily biodegradable material is in short supply and must be reserved for the PAOs.

When nitrate or oxygen is discharged to the anaerobic zone, two things may happen, both undesirable:

- They will prevent fermentation of rbCOD to acetic and propionic acid.
- Nitrates or DO could serve as electron acceptors for PAOs and other organisms that will metab-olize the VFA and so deprive the PAOs of the substance that they need to store for growth and phosphorus removal.

In the absence of electron acceptors such as DO and nitrates in the anaerobic zone, PAOs are favored to grow since they can take up and store the VFA under anaerobic conditions, thereby making it unavailable for other aerobic and facultative heterotrophs in the aerobic zone.

Biological removal of both nitrogen and phosphorus at the same WWTP is common. Both funct-ions require a carbon source. While denitrification organisms can feed on quite a number of easily degradable materials such as methanol, sugar, glucose, acetate and propionate, PAOs are restricted to the latter two for polymerization and storage (e.g. adding methanol to the anaerobic zone will reduce nitrates but not assist in the removal of phosphorus).

Current Configurations
The basic design of anaerobic, anoxic, and aerobic zones, in that order, has been achieved in many different configurations. The configurations vary in the number of stages, the nature and location of recycles, and the operation of the process. Each process was modified from the standard biological activated sludge design in order to accomplish various design goals (e.g., protection of the anaerobic zone from excess nitrate recycle). The primary processes are listed below.

Of these, all will also biologically remove nitrogen except for the Pho-redox process.
- Pho-redox (A/O)
- 3 Stage Pho-redox (A2/O)
- Modified Bardenpho
- University of Capetown (UCT) and Modified UCT (MUCT)
- Johannesburg (JHB), Modified Johannesburg, and Westbank
- Orange Water and Sewer Authority (OWASA)
- Oxidation ditches with anaerobic zones or phases added
- SBR operated with an anaerobic phase
- Hybrid chemical/biological processes

The performance of these technologies depends on many site specific factors, including but not limited to temperature, hydraulic and organic loading, recycle rates, and return streams. The technologies described in this section are generally capable of phosphorus removal to effluent levels between 0.5 and 1.0 mg/L. Operating strategies that can be used to enhance biological treatment and achieve these and, in some cases, even lower effluent levels.

Biological phosphorus removal can be combined with other technologies to achieve very low effluent concentrations (< 0.2 mg/L). Chemical addition combined with biological removal of phosphorus has been used to consistently achieve low levels. WEF and ASCE (1998) recommend that WWTPs have chemical addition capabilities even for well operating BPR plants to provide backup phosphorus removal in the event of power outages, pipe breaks, or other unforeseen events.

Solids removal can also be a limiting factor in achieving phosphorus removal below 0.2 mg/L. Very low phosphorus levels generally require a TSS level of less than 5 mg/L. Tertiary filtration (see membrane bioreactors), and advanced clarification processes can achieve TSS levels less than 5 mg/L.

Pho-redox (A/O) and 3 Stage Pho-redox (A2/O)

The Pho-redox (A/O) process is a conventional activated sludge system with an anaerobic zone at the head of the aeration basin. The RAS is pumped from the clarifier to the anaerobic zone. It is a low SRT process, operated to avoid nitrification. With no nitrates in the RAS the process is reliable and easy to operate except at temperatures in excess of 25°C when nitrification is difficult to avoid. The 3 Stage Pho-redox (A2/O) process adds an anoxic zone after the anaerobic zone to achieve de-nitrification.

In addition, a nitrate rich liquor is recycled from the end of the aerobic zone to the head of the anoxic zone to enhance de-nitrification. A shortcoming of the 3 Stage Pho-redox process is that there will be nitrates present in the RAS, potentially making the process unreliable.

Modified Bardenpho
The Bardenpho process removes nitrogen to low concentrations. The addition of an anaerobic zone at the head of the process enables phosphorus removal as well. The process consists of 5 stages: an anaerobic stage followed by alternating anoxic and aerobic stages. A nitrate-rich liquor is recycled from the first aerobic stage, designed for complete nitrification, to the first anoxic stage. The RAS is recycled from the clarifier to the beginning of the anaerobic zone. Since the nitrates in the RAS ranges from 1 to 3 mg/L, it does not seriously interfere with the mechanism for phosphorus removal as can happen in the 3 Stage Pho-redox process.

University of Cape Town (UCT) and Modified UCT (MUCT)
The UCT process was designed to reduce nitrates to the anaerobic zone when high removal of nitrates in the effluent is not required. It consists of three stages: an anaerobic stage, an anoxic stage, and an aerobic stage. The RAS is returned from the clarifier to the anoxic zone instead of the anaerobic zone to allow for denitrification and to avoid interference from nitrate with the activation of the PAOs in the anaerobic stage. A nitrate rich stream is recycled from the aerobic zone to the anoxic zone. Denitrified mixed liquor is recycled from the anoxic zone to the anaerobic zone. Several modifications of the process exist. Sometimes it can be difficult to achieve the level of denitrification in the anoxic zone required to protect the anaerobic zone from nitrates when the zone is receiving both RAS and high internal nitrate recycle flows. This problem led to the development of the modified UCT process, which splits the anoxic zone into two stages. The nitrate rich recycle from the aerobic zone is recycled to the head of the second anoxic stage. The nitrate containing RAS is recycled to the first anoxic stage where it is denitrified. Next, the denitrified RAS is recycled from the end of the first anoxic stage back to the head of the anaerobic stage and mixed with the incoming wastewater.

Johannesburg (JHB), Modified Johannesburg and Westbank
The JHB process is similar to the 3 Stage Pho-redox process, but has a pre-anoxic tank ahead of the anaerobic zone to protect the zone from nitrates when low effluent nitrates are not required. The low COD of the wastewater limited the de-nitrification capacity in the original plant (Nothern Works), resulting in nitrates in the RAS. This reduced BPR so much that a pre-anoxic tank was included on the RAS line to remove the nitrates from the RAS flow using endogenous respiration, before the flow entered the anaerobic zone. The modified JHB process adds a recycle from the end of the anaerobic zone to the head of the pre-anoxic zone to provide residual, readily biodegradable compounds for denitrification.

The Westbank process is similar to the JHB process but adds some primary effluent to the anaerobic zone to assist in denitrification with the remainder of the primary effluent being discharged to the anaerobic zone. During storm flows, excess flow is passed directly to the main anoxic zone. VFA obtained from acid fermentation of the primary sludge is passed to the anaerobic zone.

Orange Water and Sewer Authority (OWASA)
The OWASA process was developed by adding activated sludge from a biological nitrogen removal process to a trickling filter plant. Then, nitrified effluent from the trickling filter is fed to the aerobic zone of the activated sludge system. Because the VFAs have been destroyed by the trickling filter, it is necessary to ferment the settled organic solids from the primary clarifier to produce sufficient VFAs for BPR.

Next, the fermented supernatant is passed to an anaerobic (nutrition) zone and mixed with the RAS to initiate BPR. Mixed liquor then flows from the nutrition zone to an anoxic zone and then to an aerobic zone. Alternatively, simultaneous nitrification and denitrification takes place in the aeration zone.

Oxidation Ditches
There are several oxidation ditch designs that can remove phosphorus. They normally consist of an anaerobic zone ahead of the oxidation ditch whereas simultaneous nitrification and denitrification takes place within the ditches. Oxidation ditches typically operate as racetrack configurations around a central barrier, with forward mixed liquor flows of approximately 1 foot per second or more. It is possible, by manipulating the DO transferred to the mixed liquor, to establish both anoxic, aerobic and near anaerobic zones within the racetrack configuration, even though the high flow velocities accomplish complete mixing of the wastewater with the RAS.

There are many forms of oxidation ditches, such as the Carousel, the Pasveer Ditch and the Orbal process. The Orbal process creates anaerobic and anoxic zones in the outer of three concentric oval shaped ditches with the RAS recycled from the clarifier to the anoxic zone. It is also possible to introduce an anaerobic tank before the ditch to accomplish BPR in the combined system. The Pasveer Ditch and the Carousel system also can be used in conjunction with an anaerobic zone to accomplish BPR, in addition to simultaneous nitrification and denitrification within the ditches. Because of the very high internal recycle within the ditches, very low nitrate concentrations can be achieved in the mixed liquor before settling, and anaerobic conditions are easy to maintain in the anaerobic zone, thereby resulting in efficient BPR. The layout would resemble a Pho-redox process with simultaneous nitrification-denitrification (SND) in the aeration basin. Alternatively the Carousel or Pasveer Ditch could be used as the aeration stage in either the 3 Stage Pho-redox or the Modified Bardenpho process.

The VT2 process at Bowie, MD, operates two Pasveer ditches in series with dedicated anoxic, near anaerobic and aerobic zones. It also has a side stream anaerobic zone that receives only 30 percent of the influent flow to enhance BPR. Denitrified MLSS for the anaerobic zone are obtained from the end of the near anaerobic zone of the adjacent ditch. Operated without primary sedimentation, the system consistently obtains very low (<0.25 mg/L) effluent TP without chemicals or effluent filtration. The ditches are operated in series because the plant has limited clarification capacity, and series operation results in lower MLSS concentrations to the clarifiers. The biodenipho process also uses pairs of ditches.

The ditches in the biodenipho process operate in alternating anoxic-aerobic modes. An anaerobic tank is placed before the ditches for BPR and the ditches are alternated between nitrification and denitrification.

Sequencing Batch Reactors (SBR)
SBRs are fill-and-draw reactors that operate sequentially through the various phases by means of adjusting the mixing and aeration. The reactor phases can be set and automated to allow the mixed liquor to go through an anaerobic/anoxic/aerobic progression as is necessary for removal of phosphorus and nitrates. Because of the fill-and-draw nature of SBRs, it actually is necessary to remove the nitrates remaining from the previous cycle before anaerobic conditions can be established, thus the typical treatment progression becomes anoxic/anaerobic/aerobic.

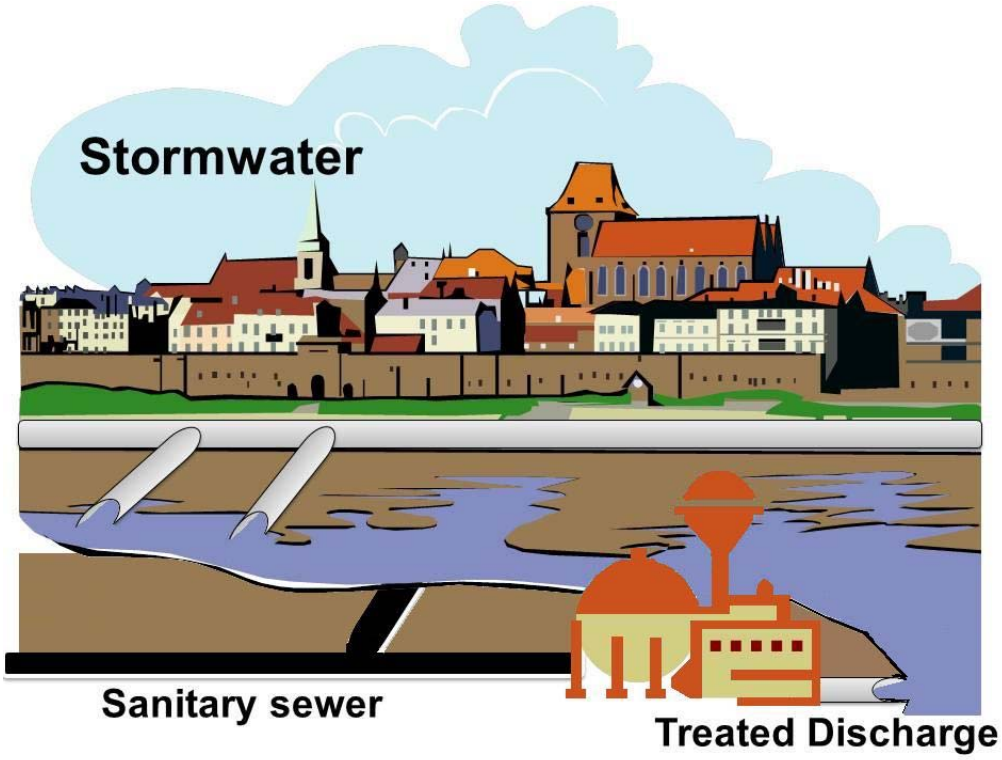
However, SBRs are almost always operated without primary sedimentation, so they still usually have a favorable BOD5:TP ratio for effluent TP of somewhat less than 1.0 mg/L during the settling phase.

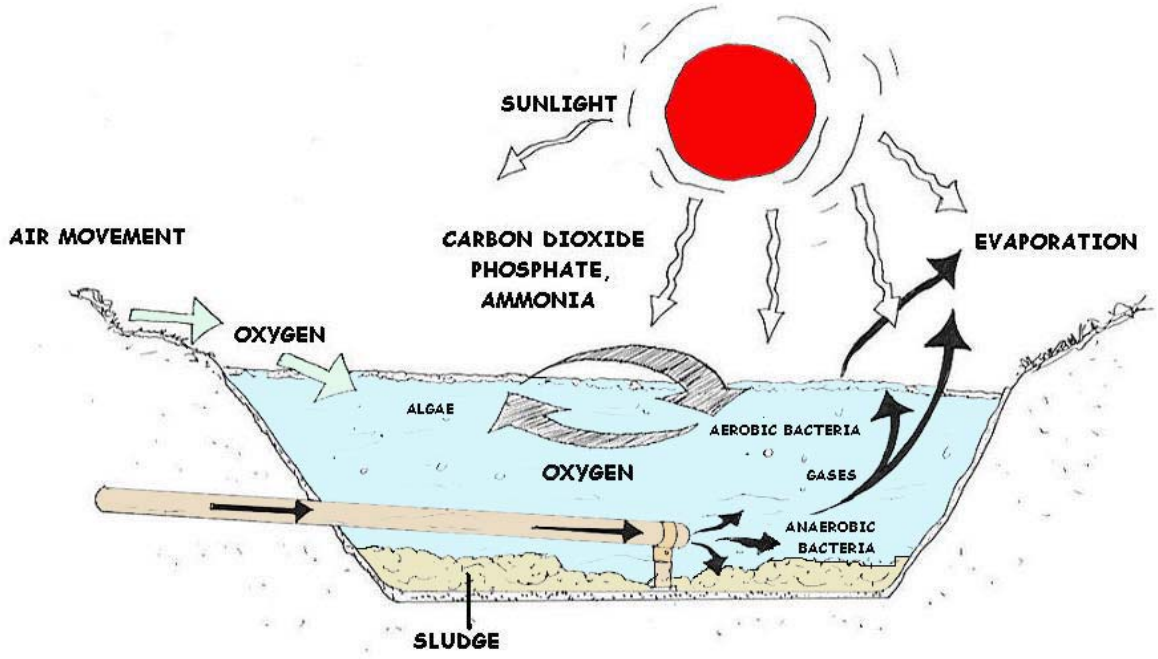
Hybrid Chemical / Biological Processes

The PhoStrip configuration, used mainly in non-nitrifying plants, pulls a side stream off the RAS in a conventional activated sludge plant. The side stream is concentrated and retained for a day or more in a thickening tank where the solids blanket is deep enough to produce anaerobic conditions and fermentation, resulting in the release of phosphates by the microorganisms. Lime is then added to the supernatant stream to precipitate and remove phosphate. The thickened, fermented sludge is passed back to the main aeration basin. Existing plants include Seneca Falls, NY; Lansdale, PA; Adrian, MI; Savage, MD; Southtowns, NY; Amherst, NY; and Reno-Sparks, NV.

The Biological Chemical Phosphorus and Nitrogen Removal (BCFS) configuration is similar to the modified UCT process. In this process, a sludge stream is removed from the anaerobic zone. Ferric chloride is added to the sludge thickener to remove phosphate. This provides an advantage over chemical addition to the secondary clarifier because it does not require the chemical sludge to be recycled. There is an existing plant at Holten in the Netherlands (WEF and ASCE, 2006), but no performance data are available.

Microscope being utilized to view activated sludge MO's. Thiothrix is a type of filament that can grow in the aeration basin of an activated sludge plant. Low DO levels are a possible cause to the growth of this long filament.

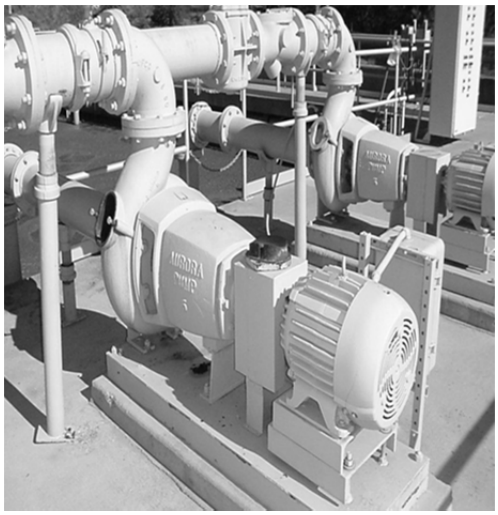




Stabilization pond - A large shallow basin used for wastewater treatment by natural processes involving the use of algae and bacteria to accomplish biological oxidation of organic matter.

Return and Waste Activated Sludge Systems

The RAS system pumps the settled sludge from the secondary clarifier back to the aeration tank. It is important that this system returns the RAS to the aeration tank before the microorganisms deplete the entire DO. The RAS must also be as concentrated as possible and the flow must be accurately measured and controlled.



To accomplish this, the RAS pumping system must have a positive variable flow control device and the RAS flow must be adjustable between the minimum and maximum range for proper process control. The desired return flow to the aeration tank could also be automatically paced to secondary influent flow.

All activated sludge processes must have a WAS system to remove excess microorganisms. This is necessary to control the F/M and MCRT. If the process is to reliably meet discharge requirements, this system must provide a positive, flexible, and reliable means of removing excess microorganisms.

It is essential for the system to have flow-metering and pumping equipment that function completely independent of other activated sludge control devices. The most positive and flexible system will include an independent pumping system with flow adjustability (for example, variable speed drive) and a flow meter that provides feedback into a flow-control device.

Such a system can be set for a given wasting rate with complete assurance that variable system head or concentration conditions will not affect its ability to remove the microorganisms required. WAS systems must have sufficient capacity to deal with both the hydraulic and/or organic load changes and process changes.

Aeration and DO Control

The purpose of aeration is two-fold: oxygen must be dissolved in the liquid in sufficient quantities to maintain the organisms and the contents of the tank must be sufficiently mixed to keep the sludge in suspension.

Mixing energy and oxygen transfer are provided through mechanical or diffused aeration. The amount of oxygen that has to be transferred by the aeration system is theoretically equal to the amount of oxygen required by the organisms in the system to oxidize the organic material.

The DO concentration in the aeration tank must be sufficient to sustain at all times the desirable microorganisms in the aeration tank, clarifier, and return sludge line back to the aeration tank. When oxygen limits the growth of microorganisms, filamentous organisms may predominate and the settleability and quality of the activated sludge may be poor.

On the other hand, over aeration can create excess turbulence and may result in the breakup of the biological floc and waste energy. Poor settling and high effluent solids will result. For these reasons, it is very important to periodically monitor and adjust the aeration tank DO levels and, for diffused air systems, the air flow rates.

In practice, the DO concentration in the aeration tank should normally be maintained at about 1.5 to 4 mg/L in all areas of the aeration tank at all times for adequate microorganism activity. Poor sludge settling as a result of filamentous organisms has been associated with mixed liquor DO concentrations below 0.5 mg/L. Above 4 mg/L, treatment usually does not significantly improve but power usage increases aeration costs considerably.

RAS Control

To properly operate the activated sludge process, a good settling mixed liquor must be achieved and maintained. The MLSS are settled in a clarifier and then returned to the aeration tank as the RAS. This keeps a sufficient concentration of activated sludge in the aeration tanks so that the required degree of treatment can be obtained in the allotted time period. The return of activated sludge from the secondary clarifier to the aeration tank is a key control parameter of the process.

The secondary clarifiers have two basic functions:

- to clarify the secondary effluent through solids/liquid separation; and
- to rapidly collect and thicken the settled solids for return to the aeration tanks or wasting to the sludge processing facilities.

Example of a Sludge Press.

Constant Rate Versus Constant Percentage Return

There are two basic ways for returning sludge to the aeration tank:

- **at a constant rate, independent of the secondary influent flow rate, and**
- **at a constant percentage of the varying secondary influent flow.**

Clarifier size and hydraulics may limit the range of practical return adjustments. Regardless of calculated values, return rates should not be reduced to the level where slowly moving, thick clarifier sludge will plug the sludge withdrawal pipes.

Also, low return rates during the night should be increased to approach the anticipated higher return rates during the day before, rather than after, the increased wastewater flows actually reach the plant. Increasing the return sludge flow after the flow increase may cause a hydraulic overload condition resulting in a carryover of solids into the clarifiers (washout).

Constant Rate Control
Returning activated sludge at a constant flow rate that is independent of the secondary influent wastewater flow rate results in a continuously varying MLSS concentration that will be at a minimum during peak secondary influent flows and a maximum during minimum secondary influent flows.

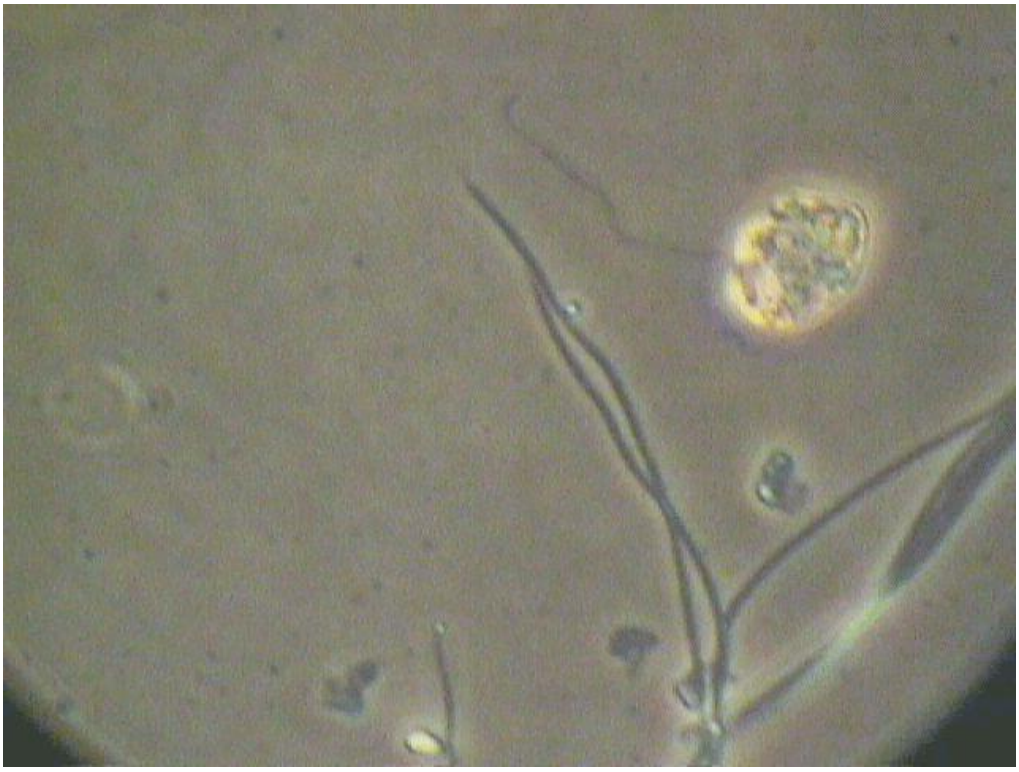
The aeration tank and the secondary clarifier must be looked at as a system where the MLSS are stored in the aeration tank during minimum wastewater flow and then transferred to the clarifier as the wastewater flow and then transferred to the clarifier as the wastewater flows initially increase.

The clarifier acts as a storage reservoir for the MLSS during periods of high flow. The clarifier has a constantly changing depth of sludge blanket as the MLSS moves from the aeration tank to the clarifier and vice versa.

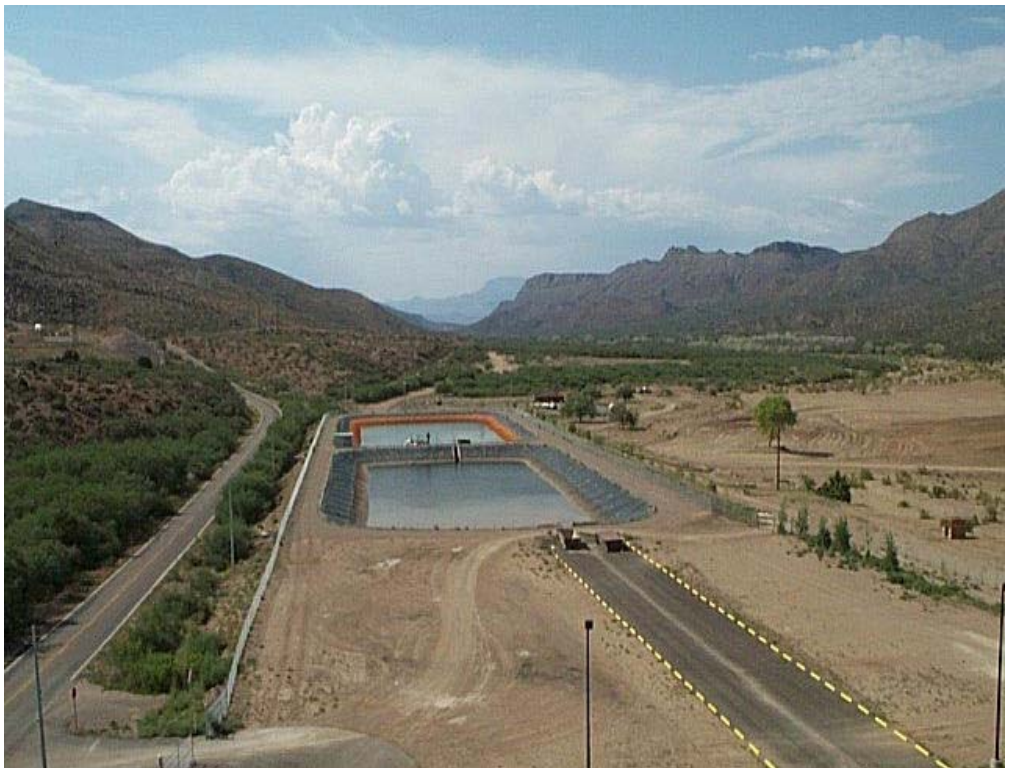
Constant Percentage Control
The second approach is to pace the return flow at a fixed percentage of the influent wastewater flow rate (Q), at a constant rate (R) R/Q. This may be done automatically with instruments, or manually with frequent adjustments.

This approach keeps the MLSS and sludge blanket depths more constant throughout high and low flow periods and also tends to maintain a more constant F/M and MCRT.

Settleability
The settleability test can be used to estimate the desirable sludge return rate. This method uses the sludge volume in a 2-L settleometer at the end of a 30-minute settling period to represent the underflow and the supernatant volume to represent the overflow.



Above photograph, Flagella.



Lagoons in series.

Rotating Biological Contactors RBC

Rotating Biological Contactors is a remediation technology used in the secondary treatment of wastewater. This technology involves allowing wastewater to come in contact with a biological medium in order to facilitate the removal of contaminants.

In its simplest form, a rotating biological contactor consists of a series of discs or media blocks mounted on a shaft which is driven, so the media rotates at right angles to the flow of sewage. The discs or media blocks are normally made of plastic (polythene, PVC, expanded polystyrene) and are contained in a trough or tank so that about 40% of their area is immersed.

The biological growth that becomes attached to the media assimilates the organic materials in the wastewater. Aeration is provided by the rotating action, which exposes the media to the air after contacting them with the wastewater. The degree of wastewater treatment is related to the amount of media surface area and the quality and volume of the inflowing wastewater.

Rotating Biological Contactors can be supplied as part of an integral package plant to treat sewage from various communities. Integral units are provided in sizes of up to a 500-population equivalent. A smaller version is also available for small private installations.

Modular systems can also be adapted to cater to populations of any number.

Multiple units have been used for populations in excess of 5000.

Each plant is designed to meet the specific requirements of the site and the effluent quality required.

Key Advantages

- **Short contact periods are required because of the large active surface.**
- **Capable of handling a wide range of flows.**
- **Sloughed biomass generally has good settling characteristics and can easily be separated from the waste stream.**
- **Operating costs are low, as little skill is required in plant operation.**
- **Retention times are short.**
- **Low power requirements.**
- **Low sludge production and excellent process control**.

Problems

White biomass over most of a RBC disc can be resolved by increasing the age of the sludge.

RBC Principles

The principles of the rotating biological contactor originated in the early 1900's but its application to sewage treatment did not occur until the 1960's when the present system was developed. The process employed relies on the well-established principle of biological oxidation using naturally occurring organisms to ensure even the most stringent effluent standards can be achieved.

Primary Settlement Zone

Rotating Biological Contactors

Incoming flows of crude sewage enter the RBC primary settlement zone, which is designed to have a buffering capacity of balancing flows up to 6 mgd (million gallons a day).
Settlement solids are retained in the tank's lower region while the partially clarified liquor passes forward to the biozone where it makes contact with the slowly rotating disks.

Contactors

Installation of Rotating Biological Contactors

Rotating Biological Contactors are available in sizes from 1100mm diameter up to 3800mm in diameter. The media packs that form the rotors are manufactured from vacuum formed black polyethylene sheets supported on the central shaft with a galvanized steel framework. The central shaft is manufactured from mild steel tube, protected internally against corrosion and fitted with end stub shafts, which are supported on split bearings.

Gearbox and Drive mechanism

Rotation is provided by a shaft mounted gearbox and motor fitted at one end.

Biozone
The rotor assembly is suspended within the biozone with 40% of the diameter submerged in the liquor at any one time. The disks slowly rotate and the continuous alternate exposure to air and sewage results in a growth of organisms known as biomass which adheres to the disks. These organisms occur naturally in the sewage and carry out the purification process by feeding off the impurities present in the sewage. As they have a short life cycle, these organisms are continually shearing off the rotating disks and pass from the biozone to the final zone.

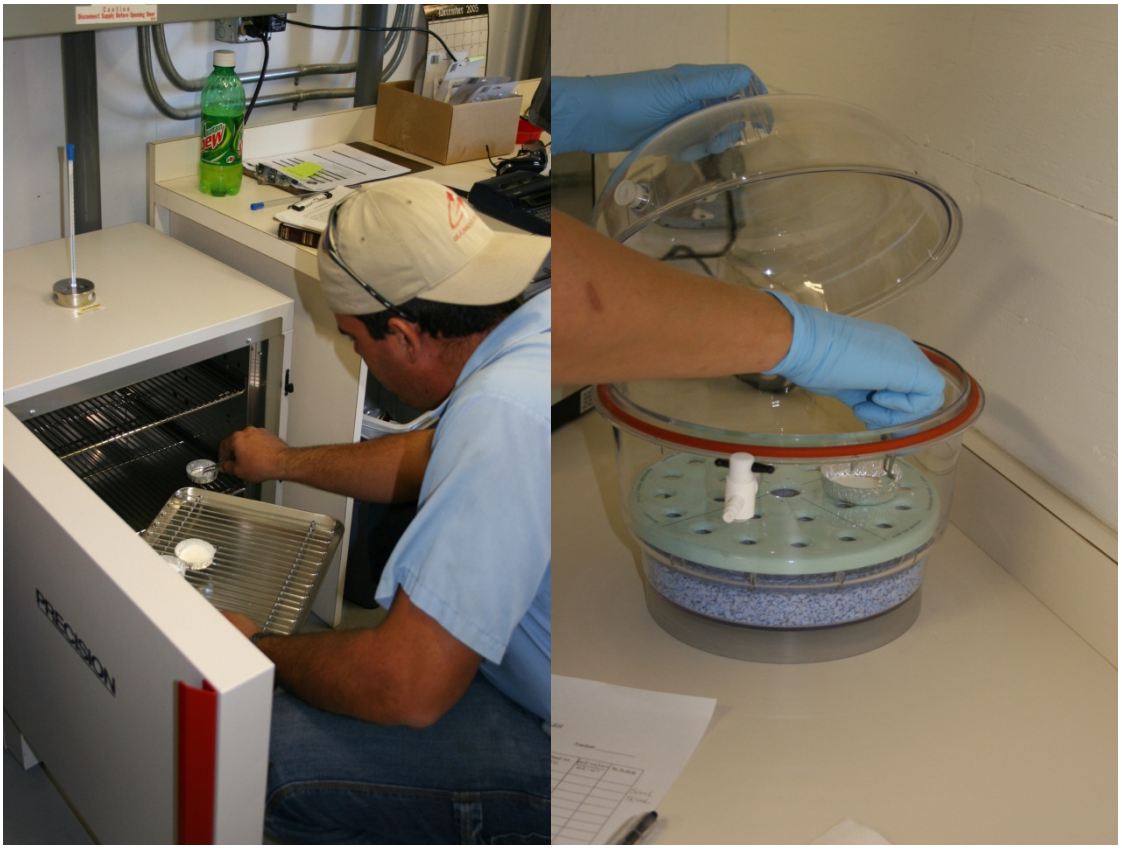
The biozone is fitted with a series of baffles between each bank of media to prevent short circuiting and to ensure maximum performance.

Final Settlement Zone

The recently completed installation at Culbokie, for Scotland Water.

The biomass passes from the biozone into the final settlement zone where it settles to form humus sludge. This is then regularly pumped out using either an air lift system or submersible pumps and returned to the primary zone.

The clarified liquid decants from the top of the tank as effluent that can be discharged to a reed bed for further clarification or direct to a watercourse.



Top left, filters being baked at 105^{o}C. Right photograph, filters in desiccant. Bottom photograph, preparation for the fecal test.

Emerging Technologies

Many plants that are not specifically configured for BPR nevertheless achieve phosphorus removal to less than 1 mg/L. The first such observation in a nitrifying plant was in a four-stage Bardenpho plant where mixed liquor was recycled from the second anoxic zone to an unstirred fermenter, then returned to the anoxic zone. The CATABOL™ and Cannibal Processes claim to reduce excess secondary sludge production by passing mixed liquor or RAS through an anaerobic (fermenting) stage and then back to the main stream aeration system. In addition, both processes pass the mixed liquor through a process for removal of some of the inert solids. Both processes claim to get similar phosphorus removal to that for the Bardenpho plant described above.

All of these processes rely on the fermentation of some of the mixed liquor for producing VFA that assists in the biological removal of phosphorus. The Town of Cary, NC, has been using a system by which some of the sludge in the return streams of a biological nitrogen removal plant is subjected to anaerobic conditions similar to that of the other processes described above resulting in an effluent phosphorus concentration of less than 0.5mg/L.

There is a similarity between these processes and *ad hoc* processes for switching off aeration in plug-flow plants for promoting phosphorus removal. These *ad hoc* processes take various forms. The Piney Water, CO, plant is a 5-stage Bardenpho plant with no primary sedimentation and little VFA in the influent, which resulted in little phosphorus removal. By switching off a mixer in one of the anaerobic zones, sludge settled to the bottom and fermented, which supplied the VFAs for reducing the orthophosphorus to less than 0.2 mg/L.

A similar operation at the Henderson, NV, plant in a JHB type process had the same effect. Some plug-flow aeration plants succeeded in reducing phosphorus to below 1 mg/L by turning off aeration at the feed end of the plant, such as the Blue Lakes and Seneca plants operated by the Metropolitan Council Environmental Service in Minnesota and the St. Cloud, MN, plant.

The Joppatowne plant operated by Harford County, MD, consists of an MLE plant with some sludge accumulation in the anoxic zone while reducing the phosphorus from 7 mg/L in the influent to around 1 mg/L in the effluent. All of these plants use the same principle of fermenting some of the mixed liquor sludge or underflow from the final clarifiers, either inside the main stream tanks or in a side stream basin. There are many instances where enterprising operators can achieve 80 percent or more phosphorus removal by turning off air or mixers in conventional treatment plants. There is a Catabol plant in Cartersville, GA (USEPA, 2008a); however, there are no published data for this plant.

Operational and Design Considerations

Important factors that affect BPR include:

- Bioavailable COD:P ratio in the anaerobic zone influent, including adjustments by VFA addition and sludge fermentation
- SRT and HRT
- Presence of oxygen or nitrate in the anaerobic zone
- Backmixing of oxygen
- Temperature
- pH
- Secondary release under anaerobic conditions
- Sufficient oxygen in the aerobic zone
- Inhibition
- Flow and load balancing

COD:P Ratio

The PAOs need VFAs in the form of acetic and propionic acid. These acids may be in the feed or can be produced through fermentation of soluble rbCOD such as sugar, ethanol, etc., in the anaerobic zone. As a rough estimate of the propensity for phosphorus removal to an effluent concentration less than 1.0 mg/L, the COD:P ratio typically should be about 40 or more. VFA is produced through fermentation of municipal wastewater or it can be added as a commercial or waste product. Some wastewater collection systems that are relatively flat and have long collection times may have sufficient fermentation in the collection system to provide the necessary VFAs, but it will vary monthly depending upon the temperature and flow conditions in the collection system. Force mains are excellent fermenters for the production of VFA. Systems that do not have a COD/P ratio of at least 40 will most likely need to supplement VFAs to achieve effluent phosphorus concentrations below 1.0 mg/L. However, they will still achieve substantial BPR with lower ratios if appropriately operated. See below for a more detailed discussion of VFAs.

Recent studies suggest that the instantaneous COD:P ratio is more important than the overall average (Neethling et al., 2005). Short term drops in the BOD:P ratio in the primary effluent to below that required for sustainable phosphorus removal correlated well with rises in effluent phosphorus. Intermittent recycles of phosphorus rich return streams may cause short term variability in the BOD:P ratio. Controlling or eliminating these recycles can improve plant performance. Weekend changes in the BOD:P ratio also can affect performance.

Another group of organisms, glycogen accumulating organisms (GAOs), also has the ability to take up acetate in the anaerobic zone, not by using energy in phosphate bonds but by using stored glycogen as the energy source. Under certain conditions, such as high temperatures or low phosphorus concentrations relative to the influent bioavailable COD, they may out-compete PAOs for the VFAs, which would result in less or no release of phosphorus in the anaerobic zone. This in turn will result in less or no overall phosphorus removal. GAOs use the stored energy in the form of glycogen to take up VFAs and store them as a complex carbohydrate containing poly-hydroxy valerate (PHV), instead of PHB formed with poly-phosphorus as the energy source. When this begins to happen, there is a slow decline of phosphorus removal by the biological system.

There is still a debate amongst researchers about the conditions likely to favor GAOs over PAOs. Summarizing a number of publications, it would appear that the following conditions favor the growth of GAOs over that of PAOs:

- High SRT
- High temperature over 28 °C
- Longer non-aerated zones
- Stronger wastes with low TKN content
- Periods of intermittent low BOD loads
- If the VFA consists mostly of either acetate or propionate
- Polysaccharides such as glucose are fed to the anaerobic zone.
- Low pH in the aerobic zone

Further confirmation is needed for some of these factors.

Volatile Fatty Acid Addition

Only VFAs such as acetic and propionic are taken up by PAOs. Reported doses of VFA for successful phosphorus removal range from 3 to 20 mg/L VFA per gram of phosphorus removed. These numbers, however, do not take into account the rbCOD that is fermented in the anaerobic zone. It is more accurate to look at the rbCOD/P ratio for good phosphorus removal, which ranges from 10 to 16. (Barnard, 2006). Surveys show that it is rare for a WWTP treating municipal sewage to achieve more than 95 percent removal of phosphorus by biological processes without adding VFAs (Neethling et al., 2005).

An Australian study shows that while both PAOs and GAOs could use acetate, PAOs will have a competitive advantage when the VFAs consist of roughly equal parts of acetic and propionic acid as a growth medium. PAOs that are fed on acetate are able to switch to propionate much more quickly and effectively than GAOs (Oehmen et al., 2005). This finding led to a strategy to feed equal amounts of acetic acid and propionic acid as the optimal for stimulating PAO growth (Oehmen et al., 2006, Bott et al., 2007). One study shows that isovaleric acid drives BPR even better than acetic acid (Bott et al.,2007).

Isovaleric acid, however, is much more expensive than acetic acid and is more odorous. It also is not significantly generated in the primary sludge fermentation process. Addition of rbCOD such as sugars and alcohols containing two carbons or more can increase phosphorus uptake by PAOs when added to the anaerobic zone but may cause sludge bulking if dosed in excess (Jenkins and Harper, 2003).

Sludge Fermentation

Anaerobic fermentation produces VFA consisting mainly of acetic and propionic acid. Some configurations, such as the Westbank and OWASA configurations, make use of anaerobic fermentation of the primary sludge to provide VFAs to the nutrient removal process. A fermentation process, however, can be added to any configuration to provide VFAs, especially in areas where little fermentation takes place in the collection system. Fermentation of the primary sludge or the RAS will produce VFA. Primary sludge fermentation is used more frequently.

There are several primary sludge fermenter designs that can accomplish this. The simplest configuration involves allowing the formation of a thicker sludge blanket in the primary clarifier itself and returning some of the thickened sludge to either the primary clarifier or to a mixing tank ahead of the primary clarifier to allow elutriation of the VFA to the primary effluent. This is referred to as an activated primary sedimentation tank (Barnard, 1984). Another variation is to pump some sludge to a complete-mix tank ahead of the primary clarifier, to accomplish fermentation. The sludge is then passed to the primary clarifier for elutriation of the VFA. Both of these processes lead to an increased load on the primary clarifier and some VFA may be lost due to aeration between the primary clarifier and the anaerobic zone. Sludge age should also be controlled to prevent methanogenic bacteria from growing and converting the VFA to methane. Usually, a SRT less than 4 days is sufficient for this.

Alternative methods accomplish fermentation in a gravity sludge thickener by holding the sludge under anaerobic conditions for 4 to 8 days. The supernatant can then be fed directly to the anaerobic zone and a high load on the primary clarifier can be avoided. Thickening can either be accomplished with a single thickener or in two stages. The two-stage process can either be a complete mix tank, followed by a thickener or two thickeners in series. It has been shown that adding molasses or other sources of readily biodegrable COD can improve the performance of fermenters (Bott et al., 2007).

RAS can also be fermented in a side stream process. The fermentation zone is similar to the anaerobic or anoxic zone of many biological processes. RAS fermentation could be used in any BPR process, but is most common in processes without primary clarifiers. Research and experience have revealed some key design considerations for primary fermenters (WEF and ASCE, 2006). These processes can have high solids content and may need a positive displacement pump to operate properly. Because fermentation can lower the pH and produce carbon dioxide and hydrogen sulfide, corrosion resistant materials should be used. Odor control may also be necessary if hydrogen sulfide is produced. Monitoring of pH and oxidation reduction potential (ORP) may be desirable to control the process.

Retention Time

The concentration of phosphorus in the sludge typically increases as the SRT increases, although the impact is very small over the SRT range of 4 to 30 days. Efficient phosphorus uptake typically requires a minimum SRT of 3 to 4 days depending on temperature. Higher SRTs will not increase phosphorus uptake, given there is sufficient VFAs available. If SRT becomes too great, however, effluent quality can degrade. This can be due to release of phosphorus as biomass degrades (WEF and ASCE, 2006). Both anaerobic and aerobic HRT can affect the amount of phosphorus stored by PAOs. Sufficient time should be allowed for the formation of VFAs and storage of the Polyhydroxyalkanoates (PHAs) in the anaerobic zone, although the reactions are relatively fast. If the time is too short, phosphorus uptake in the aerobic zone will be lower than achievable because insufficient PHAs were stored in the anaerobic zone. It has been reported that the ratio of HRT in the anaerobic zone to the HRT in the aerobic zone is important. One study found that a ratio of between 3 and 4 for aerobic HRT to anaerobic HRT led to optimal plant operation (Neethling et al., 2005).

Temperature

High temperatures can have an adverse effect on phosphorus removal. At temperatures greater than 28° C, phosphorus removal will generally be impaired, apparently by the predominance of the GAOs (Bott et al., 2007). At the low end of the temperature scale, Erdal et al. (2002) found that PAOs outcompeted GAOs at 5° C even though the PAO metabolism was slower at 5° C than at 20° C. The GAOs virtually disappeared in the 5° C reactor. Modeling studies have shown that GAOs can predominate at higher temperatures because of their increased ability to uptake acetate at those temperatures compared to PAOs (Whang et al., 2007). Low temperatures can also lower phosphorus uptake but have been shown to not be an issue in well operated and properly acclimatized plants (WEF and ASCE, 2006).

Presence of Oxygen or Nitrate in the Aerobic Zone

If oxygen or nitrate is present in the anaerobic zone, organisms that use oxygen or nitrates as electron acceptors will preferentially grow by fully oxidizing the organics to CO2 and H2O, thereby reducing the VFAs available for polymerization and storage by the PAOs. Nitrate can also inhibit fermentation of rbCOD because most of the fermenters are facultative and can use the nitrate as an electron acceptor to fully oxidize the rbCOD instead of producing VFAs as an end product of fermentation, thus depriving the PAOs of organics they can polymerize and store. Therefore, recycle of streams containing high DO and nitrate concentrations to the anaerobic zone should be avoided. Introduction of oxygen through pumps and other devices should also be avoided.

Avoiding Backmixing of Oxygen

Another potential source of oxygen and nitrates to the anaerobic zone is backmixing from downstream zones. In configurations where the anaerobic zone is followed immediately by an anoxic or aerobic zone, backmixing can cause elevated concentrations of nitrates and/or DO in the anaerobic zone leading to favoring of organisms other than PAOs. The problem can be avoided by increased baffling or changing the mixing rates. This problem is more likely to occur when the downstream zone is aerated, because aeration of mixed liquor increases the liquid depth, making the liquid level in the aerobic zone higher than in the non-aerated zone.

pH

Low pH can reduce and even prevent BPR. Below pH 6.9 the process has been shown to decline in efficiency (WEF and ASCE, 2006). This is possibly due to competition with GAOs. Filipe, et al. (2001), found that GAOs grow faster than PAOs at a pH of less than 7.25. Because many wastewater processes such as chemical addition and nitrification can lower pH, this should be monitored and adjusted if necessary. It also has been shown that it is not possible to establish enhanced biological phosphorus removal (EBPR) when the pH is less than 5.5, even though an abundant amount of acetic acid is present in the anaerobic zone (Tracy and Flammino, 1987; Randall and Chapin, 1997).

Anaerobic Release

Secondary release of phosphorus occurs when the PAOs are under anaerobic conditions in the absence of a source of VFA. The energy stored as polyphosphate is used for cell maintenance and phosphorus is released to the liquid phase (Barnard, 1984). There will then be no stored food to supply energy for the uptake of phosphorus upon subsequent aeration.

This may occur in the following process stages:
• In the anaerobic zone if the retention time is too high and the VFA is depleted well within the required retention time.
• In the main anoxic zone when that runs out of nitrates.
• In the second anoxic zone there are no nitrates to be removed.
• In the sludge blankets of final clarifiers when the RAS rate is too low and sludge is not removed fast enough.

Additionally, release may happen in aerobic zones that are too large, resulting in stored substrate depletion and destruction of PAO cells by endogenous metabolism. Since there was no food storage associated with the phosphorus release, additional carbon is then required to take up the phosphorus released, but the amount in the influent may be insufficient.

Therefore, chemicals must be added to remove the excess phosphorus. Over-design of biological nutrient removal systems could thus lead to a higher demand for an external source of VFA. Phosphorus will be released in sludge treatment processes that are anaerobic. Gravity thickening of BPR sludge can lead to phosphorus release if long retention times are used. Using mechanical dewatering instead of gravity dewatering allows less retention time and less phosphorus release (Bott et al., 2007). It is usually recommended that dissolved air flotation (DAF) be used to thicken BPR sludge to reduce the amount of phosphorus release. DAF thickening can be quite successful for the reduction of release, but if the thickened sludge is left on the DAF beach too long before removal, excessive release will occur, just as it will when the sludge is left too long in a gravity thickener.

Anaerobic digestion will also lead to phosphorus release although some phosphorus will be precipitated as either a metal salt (e.g. calcium phosphate) or as struvite (magnesium ammonium phosphate, $MgNH_4PO_4$). BPR sludge takes up and releases magnesium along with phosphates, and these two ions combine with ammonium, also present in abundance in anaerobic digesters, to form struvite.

Struvite formation is very fast, and will continue until one of the three ions is reduced to that ion's solubility level. Magnesium is usually present in the lowest concentration, and its depletion typically limits struvite formation within the anaerobic digester. Calcium phosphate precipitates also tend to form in anaerobic digesters, but they form much more slowly than struvite and the formation tends to be non-stoichiometric. If substantial amounts of phosphates are precipitated by calcium along with the struvite formation, there will be little if any propensity for struvite to form when the sludge exits the anaerobic digesters. Also, if the digested sludge is composted after dewatering, the resulting Class A sludge will be enriched in magnesium, phosphorus, nitrogen, and, to a lesser extent, potassium, which also is taken up and released with phosphorus by PAOs. Thirty percent of the phosphorus entering the anaerobic digesters at the York River plant during BPR experimentation was recycled back to the headworks from belt filter press dewatering (Randall et al., 1992).

Alternatives to anaerobic digestion such as composting, drying, or alkaline treatment can be used to reduce phosphorus release. There have been several studies which have examined using struvite precipitation as a way of recovering phosphorus from supernatant from digesters.
When anaerobic release of phosphorus occurs, recycling these streams can overload phosphorus removal processes. The effect can be worsened when the waste handling process is only operated intermittently. In some instances there is a high degree of phosphorus precipitation in the anaerobic digesters and with sufficient VFA in the influent the returned phosphorus may be removed. However, in most circumstances, some chemicals need to be added to the return streams or to the anaerobic digester itself so that the metal precipitate will be removed with the dewatered sludge.

Sufficient Oxygen in the Aerobic Zone
It is necessary for oxygen to be present in the aerobic zone for phosphorus to be taken up and retained in the activated sludge. Maintaining a sufficiently high DO transfer in the aerobic zone enhances process stability and has been found to be a key factor in phosphorus removal. (Bott et al., 2007)

Inhibition
EBPR, like any biological process, can be inhibited by chemicals toxic to the organisms. Although not as sensitive to inhibition as nitrification and rare in practice, the BPR process can be inhibited by toxic chemicals, including high concentrations of acetate (Randall and Chapin, 1997).

Flow and Load Balancing
Flows and loads to wastewater treatment plants can vary widely because of regular diurnal use patterns and because of larger, more irregular disturbances such as storm events. Peaks in either flow, or nutrient load can stress the system and cause poor performance. Peaks can be evened out using equalization tanks to balance the flow. Equalization tanks in combination with nutrient sensors can also be used to balance nutrient loads. In this case, recycle streams high in nutrient concentrations such as digester supernatant can be stored during peak nutrient loads and recycled during times when concentrations are lower.

Impacts on Sludge Handling and Removal
Stored phosphorus adds dry weight to the sludge; however, the actual PAO VSS production will be less because the reaction is less efficient than heterotrophic metabolism using DO as the electron acceptor. Sludge from BPR will be similar to sludge from conventional activated sludge plants, although it will have a higher phosphorus content. Varying results have been found with some plants reporting little or no change in settling and dewatering (Knocke et al., 1992) and others reporting enhanced settling and dewatering properties (Bott et al., 2007). The sludge produced from the process will also have higher magnesium and potassium concentrations due to co-uptake of these elements with phosphorus.

Struvite can precipitate in anaerobic processes. With abundant phosphorus and ammonia it is usually only the magnesium that is in short supply. Some magnesium is released from the digested cells with the phosphorus and may increase struvite precipitation. Some processes have proposed precipitating out struvite or other phosphate solids to avoid phosphorus return in recycle streams (Bott et al., 2007). The struvite crystals, however, depending upon where they form, can plug centrifuge ports, and pumps and pipes used to convey the sludge, if not controlled. Plugged lines are very difficult to clean.

Guidance for Selecting Process Modifications
If an existing activated sludge WWTP needs to lower phosphorus levels in its effluent, a number of options are available. Some key considerations are summarized below. For systems that do not have BPR, an anaerobic zone can be added at the head of the plant. This may be achieved by switching off aerators at the head of the reactor or by adding a separate reactor. Mixing in the anaerobic zone should be sufficient to retain biological solids in suspension without introducing oxygen. If baffling is not already present, it could be added to achieve separation of the anaerobic and aerobic zones.

Note that baffling is essential to prevent backmixing because the liquid level in the aerated zone will always be higher than that in the non-aerated zone. Therefore, an overflow baffle should be used between zones. Considerations should also be made for additional pumping needed for any recycle streams. Proper sizing of the anaerobic zone is important to ensure sufficient VFA is formed and taken up in the aeration basin. If an aerobic zone is converted to an anaerobic zone, care should be taken to ensure that the remaining aerobic zone is sufficiently sized to achieve treatment objectives. This usually is not a problem because the anaerobic zone seldom needs to be more than 15 percent of the total volume, and can be considerably less if fermentation is practiced or VFA are added. Note that much of the BOD in typical municipal sewage will be removed from solution in the anaerobic zone, and this reduces the required size of the aerobic zone, even though most of the stored BOD will be stabilized in either the anoxic or aerobic zone, or both.

For plants that already have BPR but need additional phosphorus removal, the designers should start by identifying areas that may be limiting the current process. For example, if recycle streams are intermittent, overloading of the process may occur during recycle and the process performance may suffer. Flow equalization to enable constant recycle flows may be an option in these cases. RAS when returned to the anaerobic zone may introduce nitrates or oxygen that will interfere with PAO performance. The phosphorus content of the return streams could be reduced by adding some chemicals to precipitate some of the phosphorus. Reducing oxygen introduction to the anaerobic zone from upstream processes may be needed to optimize phosphorus removal.

Plants looking to improve phosphorus removal performance should also closely examine the plant for secondary release of phosphorus. If sludge blankets in clarifiers are too deep, anaerobic
conditions can develop and cause secondary phosphorus release. This can be minimized by using deeper clarifiers, maintaining low sludge blankets, and increasing the RAS rate, so that the released phosphorus is pumped from the bottom of the clarifier rather than flowing over the effluent weir. Sludge handling can also cause excessive phosphorus release such as in gravity thickeners, DAFs and anaerobic digesters. If supernatant from these processes when poorly managed is recycled, it can overload the process. Options in this case would be to eliminate the recycle, improve operation of the process, change the process, or treat the recycle stream to remove phosphorus before it is returned to the plant.

Another area to examine in seeking improved phosphorus removal is the COD:P ratio. If the ratio is low, supplementing the current process with VFAs may provide additional removal. VFAs can either be added as a chemical addition process or produced through fermentation of primary or secondary sludge.

Other ways of improving TP removal include filtration and chemical addition. Phosphorus is often attached to colloidal particles and very low phosphorus levels usually require removal of TSS. Membrane bioreactors (MBR) in combination with biological and/or chemical phosphorus removal can result in very low effluent levels due to enhanced solids removal. Chemical addition with or without filtration can also achieve low phosphorus levels.

Effluent Filtration

Effluent filtration in combination with chemical precipitation can be used to remove phosphorous down to very low levels (< 0.1 mg/L). USEPA Region 10 (2007) found that 2-stage filtration through use of a first and second stage filter or by providing tertiary clarification prior to filtration,
resulted in the lowest effluent phosphorus concentrations of 23 WWTPs evaluated. Effluent filtration can also be used to remove soluble organic nitrogen that is not removed through biological treatment or settling.

A wide variety of filter types have been used for wastewater treatment, including:

- Conventional down-flow filters
- Deep-bed down-flow filters
- Continuous backwashing upflow sand filters
- Pulsed bed filters
- Traveling bridge filters
- Fuzzy filters
- Discfilter
- Cloth media disk filters
- Membranes
- Blue PROTM process
- Pressure filters

NO^2/NO^3, Fluoride, Sulfide, Metals, BOD-TDS-TSS
Wide-mouth Sludge/Metals bottle.

Types of Filters

This section describes the various filters listed on above page, presents key design and operating principles, and summarizes ongoing research and emerging technologies in this area.

Conventional Down-flow Filters
These filters consist of fixed-media beds typically up to 3 feet in depth and are similar to filters used to treat drinking water. Media can be single media, dual media, or multi media. Single media is typically sand or anthracite. Dual media combines anthracite and sand. Multi-media filters include a layer of garnet or limonite. Flow in these filters is by gravity from the top down. Most of the removal occurs in the top few inches of the media. The filter must be taken off-line periodically to backwash the filter to prevent clogging and too high of a pressure loss.

Deep-bed Down-flow Filters
These filters are similar to conventional down-flow filters but have deeper beds and larger media size. This gives the advantage of longer run times between backwashes. The size of the media is limited by the ability to backwash the filter. Because these filters are more difficult to backwash, air scour is necessary to fully clean the filter bed.

Continuous Backwashing Upflow Sand Filters
During operation of the continuous backwashing upflow filter, water is introduced through risers at the bottom of a deep sand bed. Water flows upward through the sand bed and over an overflow weir. Sand and trapped solids flow downward through the filter and are drawn into the suction of an airlift pipe in the center of the filter. As the sand travels up the airlift pipe, energy from the air scours the particles and separates the sand from filtered solids. At the top of the airlift pipe, the clean sand settles back onto the top of the filter and the solids are carried away into a reject line.
These filters have the advantage of having no moving parts other than the air compressor and requiring less energy and maintenance than traditionally backwashed filters. They are sometimes referred to by the trade name Dynasand.

Pulsed Bed Filters
Pulsed bed filters are shallow filters with an unstratified fine sand media. An air pulse disturbs the media and allows penetration of solids into media bed, allowing the entire filter bed to be used for removal of solids. The pulse is designed to expand the filter operation and reduce the number of backwash cycles, although the filter must still be periodically backwashed to remove the solids.

Traveling-Bridge Filters
Traveling-bridge filters consist of long shallow beds of granular media. Wastewater is applied to the top of the media and flows downward. Each cell is individually backwashed by a traveling-bridge while the other cells continue to operate. The bridge uses filtered water to backwash the filters and includes surface wash to breakup matted solids or clumps of solids.

Fuzzy Filters
The fuzzy filter uses a proprietary synthetic filter media that is highly porous. Water flows not only around the media but also through it, allowing much higher filtration rates. The media is held in place by a metal plate and flow is from the bottom of the bed upwards. The filter is backwashed by raising the plate and introducing a horizontal air stream from alternating sides causing the media to roll back and forth. The effluent is returned to the plant.

Discfilters
Discfilters are a series of parallel mounted disks used to support a cloth filter media. Water enters a central tube and flows out between the two layers of cloth in each disk. The disks rotate and are normally 60 to 70 percent submerged. The portion above the water is backwashed using spray nozzles.

Cloth Media Disk Filters
The cloth media disk filter is similar to the discfilter listed above. In this case the water flows from the outside of the partially submerged cloth disks and into a center pipe. Disks continue to rotate during backwash and water is sucked into the disc using suction heads.

Membranes
Membrane systems use a pressure head to drive water through a permeable membrane. Membrane filters are typically classified by their pore size which in turn determines the size of the particles they exclude. Microfiltration, ultrafiltration, nanofiltration, and reverse osmosis (RO) remove increasingly smaller particles. Microfiltration and ultrafiltration remove 3 to 6 logs of bacteria, 95 percent or more BOD, along with most particles (WEF, 2006). Nanofiltration removes nearly all particles including some viruses. RO removes all particles as well as most large dissolved constituents. The energy cost for applying the pressure head and the need to replace membranes make membrane filtration a more expensive technology. It can achieve very low concentrations of nutrients and other contaminants, however, and is common in water re-use projects.

Membranes can be configured a number of ways including hollow fiber, spiral wound, plate and frame, cartridge, or in pressure vessels. Membranes can foul from organics, biological activity, or metals in the wastewater. Typically the water must be pre-treated before using these membranes. Pretreatment could be conventional filters, cartridge filters, or larger membrane filters. Disinfection may also be required to prevent biological fouling.

Blue PROTM Process
The Blue PROTM process uses a continuous backwashing filter that is designed remove phosphorus. Filters can be run in series for even greater removal. The filter media (sand) is coated with a hydrous ferric oxide coating, which enhances phosphorus removal through adsorption. A ferric salt is added prior to the filter to aid in coagulation and to replace the ferric coating which is abraded from the sand. Water flows up through the filter while the sand travels down. An airlift tube at the bottom of the filter carries the sand upward. Turbulence from the compressed air knocks accumulated iron and phosphorus along with any solids off the particle as it travels upward. The iron, phosphorus, and particles are wasted, while the clean sand is deposited on the top of the bed. The filters can be run biologically active to achieve denitrification.

The Blu-CAT process combines the Blu-Pro process with addition of advanced oxidants. Early pilot tests show that this process is capable of removing other emerging contaminants along with phosphorus and microorganisms (USEPA, 2008a).

Pressure Filters
Pressure filters are similar to conventional media filters except they are contained in closed containers and are filtered under pressure. The increased pressure creates a greater head loss and allows longer times between backwashes.

Design and Operating Principles
Filtration is mainly affected by the concentration and size distribution of particles entering the filter. Turbidity is often used as a surrogate for particle concentration. The concentration of particles will affect run-time in filters and will also affect the required surface area to achieve the desired filtration. The size distribution of the particles and its relevance to pore size of the granular or membrane filters will affect the removal mechanisms. Filtration rate is also an important design parameter. Too fast of a filtration rate can cause floc to break up and pass through the filter. The optimal filtration rate depends on floc strength, which in turn depends on the biological treatment processes prior to filtration (e.g., Higher SRTs lead to weaker flocs). The filtration rate, along with the loading rate will determine the area of the filter required. The higher the loading rate, the more frequent backwashes will be required and the greater the head loss across the filters. Typical filtration rates are 5 to 15 meters per hour for gravity filters and up to 20 meters per hour for pressure filters (WEF and ASCE, 1998).

Addition of polymers or other coagulant aids can greatly aid filtration. Typical doses for filter influent are 0.05 to 0.15 mg/L of organic polyelectrolyte (WEF and ASCE, 1998), although jar tests are conducted to determine the proper dose. Too low a dose can allow uncoagulated particles through the filter and too high a dose can lead to mudballs and filter clogging.

There are several ways the flow rate can be controlled in filters. Constant-rate fixed head filtration maintains a constant flow through the filter. This will lead to an increased head above the filter as the filter run progresses. In constant-rate variable head filtration the rate is kept the same and the filter is backwashed when the head reaches a certain value. In variable-rate filtration, the rate of filtration decreases throughout the filter run until it reaches a minimum value and is backwashed.
Variable-rate filtration is less common than constant-rate filtration.

Proper backwashing is also important to filter operation. Without proper backwashing there can be breakthrough of particles and turbidity. Lack of a proper backwash can also lead to accumulation of materials on the surface of the filter that can form mudballs and cracks, which can allow solids to pass through the filter. A surface wash or air scour may also be helpful to prevent accumulation of mudballs or grease. Surface wash or air scour is also helpful for traveling bridge filters. Without surface wash traveling bridge filters are limited to an influent TSS concentration of 40 to 50 mg/L (WEF and ASCE, 1998).

If membrane filters are used, fouling can be an important consideration. Cellulose acetate membranes can be damaged by biological activity. Disinfection is often used to prevent biological
fouling of the membranes. Some membrane materials such as polyacramides, however, can be damaged by chlorine. This can be avoided by using an alternative disinfectant, a different membrane material, or by de-chlorination. Lowering the pH can help to prevent mineral fouling of nanofiltration or reverse osmosis membranes. Besides pre-treatment, chemical cleaning of the membranes may also be required periodically. Monitoring of effluent quality and pressure differential can be important to help identify membrane fouling or failure.

Ongoing Research and Emerging Technologies
The use of membranes as tertiary filtration is an area that has recently expanded. Research continues on various membrane configurations along with topics such as pre-treatment, membrane cleaning, and removal of emerging contaminants. Fuzzy filters are also an innovative technology that is beginning to be established in the wastewater community with several full scale projects.

Other research has focused on enhancements to existing technology. For example, the Blue-Pro system combines continuous backwashing filters, a well-known technology, with a hydrous ferric oxide coating and ferric salt addition to remove phosphorus by adsorption as well as filtration.

Mathematical Modeling
8.1 The Need for Models
WWTPs are complex systems that depend on numerous biological, chemical, and physical processes to achieve effluent goals. Because of the complex behavior of the processes and the
variability in wastewater characteristics, biological populations, and plant design, it is not always
possible to predict how changing any one variable will affect the effluent quality.

Plant designs that work for one influent wastewater and climate may not perform well in different conditions. Pilot scale or full scale trials can help to determine the effect of various parameters, but costs and time to cover all possibilities may be prohibitive. Therefore, models fill an important need by enabling simulation of a process and estimating the impact that changing parameters will have on the treatment effectiveness.

Models can be used for a number of purposes including the design of new WWTPs, the design of retrofits or upgrades to existing plants, determining how changes in operations may affect effluent concentrations of permitted contaminants, determining how plants will respond to changes in influent quality or flow, and for training operators. Not all models can achieve all of these purposes, so models should be selected with the desired use in mind. There is some disagreement in the literature in the use of the term model.

Some references use the term to refer to sets of mathematical equations that characterize a process, other references use model to refer to the computer program used to solve these equations. This section will use the former and will use the term “simulator” to describe the computer program.

Overview of Available Models

Models are sets of equations, generally based on theory and grounded in empirical data that represent a wastewater treatment process. Each unit process is represented by its own model.

Model equations for processes such as clarification and settling are well known and fairly simple. Modeling biological wastewater processes such as activated sludge, however, is much more complicated. The primary set of models for activated sludge processes has been compiled by the International Water Association (IWA). The first model was developed in 1986 and was called the activated sludge model (ASM). Later known as ASM1, this model was able to model the biological oxidation of carbon, nitrification, and de-nitrification.

Although the ASM model gained widespread use among both academia and industry, it had limitations. For example the model assumed constant temperature and pH, did not include EBPR, and the biological reactions did not depend on the carbon source.

In order to improve the model, IWA developed four other ASM models; ASM2, ASM2d, ASM3, and ASM3 with BioP. ASM2 and ASM2d were intended to add EBPR. The ASM3 models were intended to deal with limitations such as the independence of the ASM1 model of temperature and carbon source. In addition, other models were developed to seek to improve upon the ASM model.

The metabolic biological phosphorus model of the Delft University of Technology (TUDP) was developed to fully account for the metabolism occurring in PAOs during EBPR. Barker and Dold (1997) developed a model (B&D) to include different rates of growth depending on the carbon source.

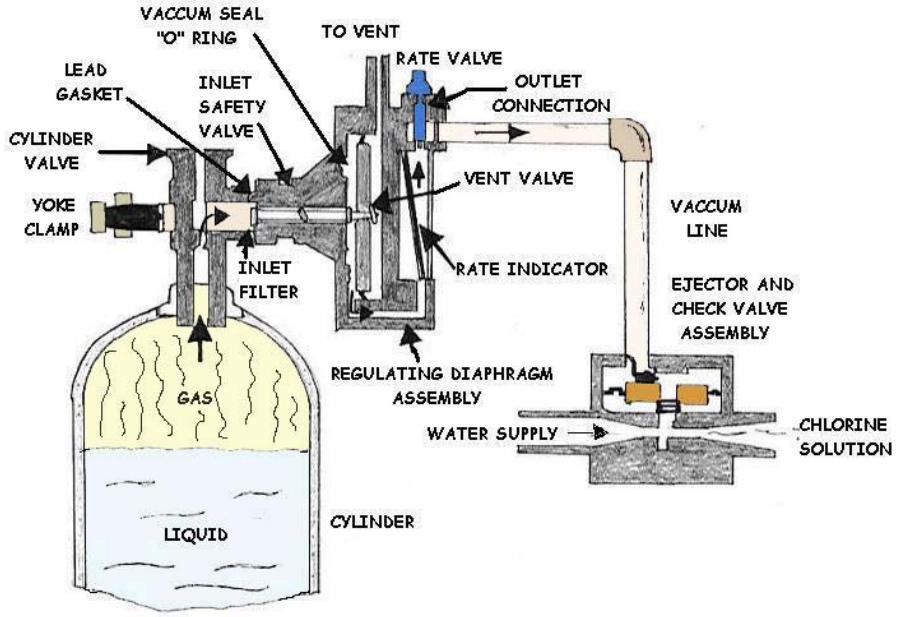
When selecting models, the processes required and the range of normal operating parameters for the plant should be considered and compared to the available models. For example, if chemical phosphorus removal is to be used in a plant, the plant is limited to using either the ASM2 or ASM2d models. Each model also has a range of temperatures and pH over which it is valid.

Chlorine Section

1 Ton Containers

The top line or valve is for extracting the gas, and the bottom line is for extracting the Cl2 liquid. Never place water on a leaking metal cylinder. The water will help create acid which will make the leak larger.

150 Pound Chlorine Cylinder



Chlorine Exposure Limits and Related Information

This information is necessary to pass your pre-test.

*** OSHA PEL 1 PPM - IDLH 10 PPM and Fatal Exposure Limit 1,000 PPM**

The current Occupational Safety and Health Administration (**OSHA**) permissible exposure limit (**PEL**) for chlorine is 1 ppm (3 milligrams per cubic meter ($mg/m^{(3)}$)) as a ceiling limit. A worker's exposure to chlorine shall at no time exceed this ceiling level. *** IDLH 10 PPM**

Physical and chemical properties of chlorine: A yellowish green, nonflammable and liquefied gas with an unpleasant and irritating smell. Can be readily compressed into a clear, amber-colored liquid, a noncombustible gas, and a strong oxidizer. Solid chlorine is about 1.5 times heavier than water and gaseous chlorine is about 2.5 times heavier than air. Atomic number of chlorine is 17. Cl is the elemental symbol and Cl2 is the chemical formula.

Monochloramine, dichloramine, and trichloramine are also known as Combined Available Chlorine. $Cl_2 + NH_4$.

HOCl and OCl-; The **OCL-** is the hypochlorite ion and both of these species are known as free available chlorine. These are the two main chemical species formed by chlorine in water and they are known collectively as hypochlorous acid and the hypochlorite ion. When chlorine gas is added to water, it rapidly hydrolyzes. The chemical equation that best describes this reaction is **$Cl2 + H_2O$ --> H+ + Cl- + HOCl.** Hypochlorous acid is the most germicidal of the chlorine compounds with the possible exception of chlorine dioxide.

Yoke-type connectors should be used on a chlorine cylinder's valve, assuming that the threads on the valve may be worn.

The connection from a chlorine cylinder to a chlorinator should be replaced by using a new, approved gasket on the connector. Always follow your manufacturer's instructions.

On 1 ton Chlorine gas containers, the chlorine pressure reducing valve should be located downstream of the evaporator when using an evaporator. This is the liquid chlorine supply line and it is going to be made into Chlorine gas.

In water treatment, chlorine is added to the effluent before the contact chamber (before the clear well) for complete mixing. One reason for not adding it directly to the chamber is that the chamber has very little mixing due to low velocities.

Here are several safety precautions when using chlorine gas. In addition to protective clothing and goggles, chlorine gas should be used only in a well-ventilated area so that any leaking gas cannot concentrate. Emergency procedures in the case of a large uncontrolled chlorine leak are as follows: Notify local emergency response team, warn and evacuate people in adjacent areas, and be sure that no one enters the leak area without adequate self-contained breathing equipment.

Here are several symptoms of chlorine exposure. Burning of eyes, nose, and mouth, coughing, sneezing, choking, nausea and vomiting, headaches and dizziness, fatal pulmonary edema, pneumonia, and skin blisters. A little Cl_2 will corrode the teeth and then progress to throat cancer.

Approved method for storing a 150 - 200 pound chlorine cylinder: Secure each cylinder in an upright position, attach the protective bonnet over the valve and firmly secure each cylinder. Never store near heat. Always store the empty in an upright, secure position with proper signage.

Chlorine Timeline

1879
- This marked the first time that chlorine was applied as a disinfectant. William Soper of England treated the feces of typhoid patients before disposal into the sewer. He used chlorinated lime, which was a common form of chlorine used initially. (White, 1972)

1893
- This date was the first time that chlorine was applied as a disinfectant on a plant scale basis. This application was made at Hamburg, Germany. (White, 1972)

1903
- This marked the first time chlorine gas was used as a disinfectant in drinking water. This took place in Middlekerke, Belgium. Prior to this date, chlorine was applied through the use of hydrated lime, chloride of lime, or bleaching powder. The use of chlorine gas was designed by Maurice Duyk, a chemist for the Belgian Ministry of Public Works. (Pontius, 1990)

1908
- The first full scale chlorine installation at a drinking water plant in the United States was initiated in this year. This installation took place at the Bubbly Creek Filter Plant in Chicago. This plant served the Chicago Stockyards and was designed by George A. Johnson. The raw water contained a large amount of sewage which was causing sicknesses in the livestock. Johnson implemented chlorine through chloride of lime, and the bacterial content of the water dropped drastically. (Pontius, 1990)

1910
- C. R. Darnall became the first to use compressed chlorine gas from steel cylinders, which is an approach still commonly used today. His installation was in Youngstown, Ohio. His implementation used a pressure-reducing mechanism, a metering device, and an absorption chamber. It was moderately successful, but his setup was only used once.

1912
- John Kienle, chief engineer of the Wilmington, Delaware water department, invented another way to apply chlorine to drinking water. He developed a way to push compressed chlorine from cylinders into an absorption tower in which water was flowing opposite the flow of the chlorine. Because the gas flow was opposite the water flow, the chlorine was able to disinfect the water. (Pontius, 1990)

1913
- An Ornstein chlorinator was installed at Kienle's Wilmington, Delaware water treatment plant. This marked the first time a commercial chlorination system was installed at a municipal water treatment plant. The chlorinator used the same basic premise that Kienle's previous installation did, but the Ornstein chlorinator used both a high and low pressure gauge to more accurately control the amount of chlorine added to the system. (Pontius, 1990)

1914
- On October 14, 1914, the Department of the Treasury enacted the first set of standards that required the use of disinfection for drinking water. These standards called for a maximum level of bacterial concentration of 2 coliforms per 100 milliliters. Because chlorination was the main disinfectant at the time, these standards dramatically increased the number of treatment plants using chlorine. (White, 1972)

1919
- Two important discoveries were made during this year. Wolman and Enslow discovered the concept of chlorine demand which states that the amount of chlorine needed to disinfect the water is related to the concentration of the waste and the amount of time the chlorine has to contact the water. The other important discovery of 1919 was by Alexander Houston. He discovered that chlorine can also eliminate taste and odor problems in water. (Pontius, 1990)

1925
- New drinking water standards were enacted that reduced the maximum permissible limit of coliforms from 2 to 1 coliform per 100 milliliters. This increased the amount and frequency of chlorination again. (White, 1972)

1939
- The theory of the chlorine breakpoint was discovered in this year. Chlorine breakpoint theory is discussed in the following section. (White, 1972)

1960
- A new implementation practice was discovered in this year. The compound loop principle of chlorinator control was implemented, which is the most recent major discovery in chlorine application. (White, 1972)

1972
- A report entitled "Industrial Pollution of the Lower Mississippi River in Louisiana" was published containing the first evidence of disinfection byproducts in drinking water resulting from organic pollution in source water. (Pontius, 1990)

As is evident by the dates in the timeline, most of the innovation in chlorination occurred over 70 years ago. Very few innovations or discoveries have been made recently. Most of the current research is being performed in other areas of disinfection. These areas include ozone, chlorine dioxide, and UV radiation. Chlorine is still the most widely used disinfectant in the United States, but other areas of the world are beginning to use other methods of disinfection with increasing frequency. Since chlorine is still widely used, a thorough understanding of how it disinfects and is implemented is important to those interested in water treatment.

Chlorine Supplement **Pre-Quiz**

1. How should the connection from a chlorine cylinder to a chlorinator be replaced?

2. How many turns should a chlorine gas cylinder be initially opened?

3. If the temperature of a full chlorine cylinder is increased by 50°F or 30°C, what is the most likely result?

4. What is meant by the specific gravity of a liquid?

5. Which metals are the only metals that are ***TOTALLY*** inert to moist chlorine gas?

6. What will be discharged when opening the top valve on a one-ton chlorine cylinder?

7. What are the approved methods for storing a chlorine cylinder?

8. What are normal conditions for a gas chlorination start-up?

9. Name a safety precaution when using chlorine gas?

10. What compounds are formed in water when chlorine gas is introduced?

11. Why should roller bearings not be used to rotate a one-ton chlorine cylinder?

12. What are the physical and chemical properties of chlorine?

13. What are the necessary emergency procedures in the case of a large uncontrolled chlorine leak?

14. Name several symptoms of chlorine exposure.

15. 5 lbs. of a 70% concentration sodium hypochlorite solution is added to a tank containing 650 gallons of water. What is the chlorine dosage?

16. As soon as Cl_2 gas enters the throat area, a victim will sense a sudden stricture in this area - nature's way of signaling to prevent passage of the gas to the lungs. At this point, the victim must attempt to do two things. Name them.

17. Positive pressure SCBAs and full face piece SARs can be used in oxygen deficient atmospheres containing less than what percentage of oxygen in the atmosphere?

18. Death is possible from asphyxia, shock, reflex spasm in the larynx, or massive pulmonary edema. Populations at special risk from chlorine exposure are individuals with pulmonary disease, breathing problems, bronchitis, or chronic lung conditions.
A. TRUE
B. FALSE

19. Chlorine gas reacts with water producing a strongly oxidizing solution causing damage to the moist tissue lining the respiratory tract when the tissue is exposed to chlorine. The respiratory tract is rapidly irritated by exposure to 10-20 ppm of chlorine gas in air, causing acute discomfort that warns of the presence of the toxicant.
A. TRUE
B. FALSE

20. Even brief exposure to 1,000 ppm of Cl_2 can be fatal.
A. TRUE
B. FALSE

21. What are the two main chemical species formed by chlorine in water and what name are they are known collectively as?

22. When chlorine gas is added to water, it rapidly hydrolyzes according to the reaction:

23. Which chemical reaction equation represents the dissociation of hypochlorous acid?

24. This species of chlorine is the most germicidal of all chlorine compounds with the possible exception of chlorine dioxide.

Here are both half ton container and 150 pound chlorine gas cylinders.
Answers in rear section before the final assignment.

Chlorine evaporators next to a rail tank chlorine cylinder. Bottom photograph, SCBA should always be stored on the outside of the chlorine storage room.

Chlorine C repair kit, used for the tank cars and tankers.

Chlorine Introduction

Name: Chlorine
Symbol: Cl
Atomic Number: 17
Atomic Mass: 35.4527 amu
Melting Point: -100.98 °C (172.17 K, -149.764 °F)
Boiling Point: -34.6 °C (238.55 K, -30.279997 °F)
Number of Protons/Electrons: 17
Number of Neutrons: 18
Classification: Halogen
Crystal Structure: Orthorhombic
Density @ 293 K: 3.214 g/cm^3
Color: Green
Uses: Water purification, bleaches
Obtained From: Salt
Date of Discovery: 1774
Discoverer: Carl Wilhelm Scheele
Name Origin: From the Greek word *khlôros* (green)

Chlorine Gas Information
Identifiers
1. CAS No.: 7782-50-5
2. RTECS No.: FO2100000
3. DOT UN: 1017 20
4. DOT label: Poison gas

Chlorinators

Safety Data
NIOSH IDHL: 25 ppm
NIOSH Ceiling: **0.5ppm/15 minutes**
PEL/TWA: **1 ppm**
TLV/TWA: **1 ppm**
TLV/STEL: **3 ppm**
TLV/IDLH: **25 ppm**

Physical Data
1. **Molecular weight:** 70.9
2. **Boiling point (at 760 mm Hg):** -34.6 degrees C (-30.28 degrees F)
3. **Specific gravity (liquid):** 1.41 at 20 degrees C (68 degrees F) and a pressure of 6.86 atm
4. **Vapor density**: 2.5
5. **Melting point:** -101 degrees C (-149.8 degrees F)
6. **Vapor pressure at 20 degrees C (68 degrees F):** 4,800 mm Hg
7. **Solubility**: Slightly soluble in water; soluble in alkalis, alcohols, and chlorides.
8. **Evaporation rate:** Data not available.

Chlorine's Appearance and Odor
Chlorine is a greenish-yellow gas with a characteristic pungent odor. It condenses to an amber liquid at approximately -34 degrees C (-29.2 degrees F) or at high pressures. Odor thresholds ranging from 0.08 to part per million (ppm) parts of air have been reported. Prolonged exposures may result in olfactory fatigue.

Reactivity
1. **Conditions Contributing to Instability**: Cylinders of chlorine may burst when exposed to elevated temperatures. Chlorine in solution forms a corrosive material.
2**. Incompatibilities**: Flammable gases and vapors form explosive mixtures with chlorine. Contact between chlorine and many combustible substances (such as gasoline and petroleum products, hydrocarbons, turpentine, alcohols, acetylene, hydrogen, ammonia, and sulfur), reducing agents, and finely divided metals may cause fires and explosions. Contact between chlorine and arsenic, bismuth, boron, calcium, activated carbon, carbon disulfide, glycerol, hydrazine, iodine, methane, oxomonosilane, potassium, propylene, and silicon should be avoided. Chlorine reacts with hydrogen sulfide and water to form hydrochloric acid, and it reacts with carbon monoxide and sulfur dioxide to form phosgene and sulfuryl chloride. Chlorine is also incompatible with moisture, steam, and water.
3. **Hazardous Decomposition Products:** None reported.
4**. Special Precautions:** Chlorine will attack some forms of plastics, rubber, and coatings.

Flammability
Chlorine is a non-combustible gas.
The National Fire Protection Association has assigned a flammability rating of 0 (no fire hazard) to chlorine; however, most combustible materials will burn in chlorine.
1. **Flash point:** Not applicable.
2. **Autoignition temperature**: Not applicable.
3. **Flammable limits in air:** Not applicable.
4. **Extinguishant:** For small fires use water only; do not use dry chemical or carbon dioxide. Contain and let large fires involving chlorine burn. If fire must be fought, use water spray or fog.

Fires involving chlorine should be fought upwind from the maximum distance possible.

Keep unnecessary people away; isolate the hazard area and deny entry. For a massive fire in a cargo area, use unmanned hose holders or monitor nozzles; if this is impossible, withdraw from the area and let the fire burn. Emergency personnel should stay out of low areas and ventilate closed spaces before entering.

Containers of chlorine may explode in the heat of the fire and should be moved from the fire area if it is possible to do so safely. If this is not possible, cool fire exposed containers from the sides with water until well after the fire is out. Stay away from the ends of containers. Firefighters should wear a full set of protective clothing and self-contained breathing apparatus when fighting fires involving chlorine.

Chlorine Exposure Limits

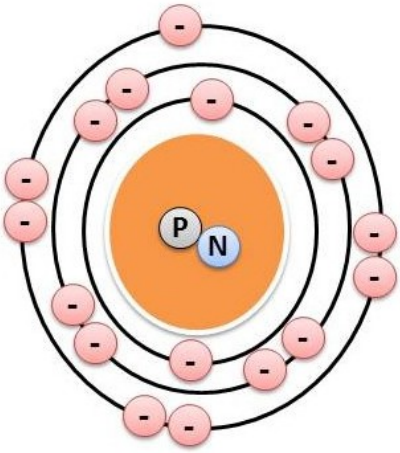
*** OSHA PEL**
The current **OSHA** permissible exposure limit (**PEL**) for chlorine is 1 ppm (3 milligrams per cubic meter ($mg/m^{(3)}$)) as a ceiling limit. A worker's exposure to chlorine shall at no time exceed this ceiling level [29 CFR 1910.1000, Table Z-1].

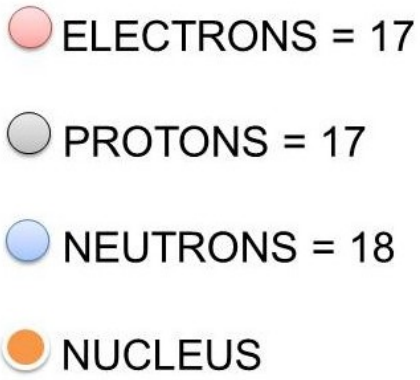
*** NIOSH REL**
The National Institute for Occupational Safety and Health (**NIOSH**) has established a recommended exposure limit (**REL**) for chlorine of 0.5 ppm $mg/m^{(3)}$) as a TWA for up to a 10-hour workday and a 40-hour workweek and a short-term exposure limit (**STEL**) of 1 ppm (3 $mg/m^{(3)}$)[NIOSH 1992].

*** ACGIH TLV**
The American Conference of Governmental Industrial Hygienists (**ACGIH**) has assigned chlorine a threshold limit value (**TLV**) of 0.5 ppm (1.5 $mg/m^{(3)}$) as a TWA for a normal 8-hour workday and a 40-hour workweek and a **STEL** of 1 ppm (2.9 $mg/m^{(3)}$) for periods not to exceed 15 minutes. Exposures at the STEL concentration should not be repeated more than four times a day and should be separated by intervals of at least 60 minutes [ACGIH 1994, p. 15].

*** Rationale for Limits**
The NIOSH limits are based on the risk of severe eye, mucous membrane and skin irritation [NIOSH 1992]. The ACGIH limits are based on the risk of eye and mucous membrane irritation [ACGIH 1991, p. 254].

Chlorine's Atomic Structure





Isotopes

Isotope	Half Life
Cl-35	Stable
Cl-36	301000.0 years
Cl-37	Stable
Cl-38	37.2 minutes

Top photograph, this blue device prevents the liquid from being pulled and freezing the lines. Bottom photograph, the application of an ammonia mist to detect a chlorine gas leak.

Chlorine Basics

Chlorine is one of 90 natural elements, the basic building blocks of our planet. To be useful, an element must be relatively abundant or have extremely desirable properties. Chlorine has both characteristics. As a result -- over the course of many decades of careful research and development -- scientists have learned to use chlorine and the products of chlorine chemistry to make drinking water safe, destroy life-threatening germs, produce life-saving drugs and medical equipment, shield police and fire fighters in the line of duty, and ensure a plentiful food supply.

In 1774, in his small experimental laboratory, Swedish pharmacist Carl Wilhem Scheele released a few drops of hydrochloric acid onto a piece of manganese dioxide. Within seconds, a greenish-yellow gas arose. Although he had no idea at the time, he had just discovered chlorine.

The fact that the greenish-yellow gas was actually an element was only recognized several decades later by English chemist Sir Humphrey Davy. Until that time, people were convinced that the gas was a compound of oxygen. Davy gave the element its name on the basis of the Greek word khloros, for greenish-yellow. In 1810 he suggested the name "*chloric gas*" or "*chlorine*."

One of the most effective and economical germ-killers, chlorine also destroys and deactivates a wide range of dangerous germs in homes, hospitals, swimming pools, hotels, restaurants, and other public places. Chlorine's powerful disinfectant qualities come from its ability to bond with and destroy the outer surfaces of bacteria and viruses. First used as a germicide to prevent the spread of "child bed fever" in the maternity wards of Vienna General Hospital in Austria in 1846, chlorine has been one of society's most potent weapons against a wide array of life-threatening infections, viruses, and bacteria for 150 years.

When the first men to set foot on the moon returned to earth (Apollo 11 mission: 24.7.69) a hypochlorite solution was chosen as one of the disinfectants for destroying any possible moon germs.

What Happens to Chlorine When it Enters the Environment?

- When released to air, chlorine will react with water to form hypochlorous acid and hydrochloric acid, which are removed from the atmosphere by rainfall.

- Chlorine is slightly soluble in water. It reacts with water to form hypochlorous acid and hydrochloric acid. The hypochlorous acid breaks down rapidly. The hydrochloric acid also breaks down; its breakdown products will lower the pH of the water (makes it more acidic).

- Since chlorine is a gas it is rarely found in soil. If released to soil, chlorine will react with moisture forming hypochlorous acid and hydrochloric acid. These compounds can react with other substances found in soil.

- Chlorine does not accumulate in the food chain.

Disinfectant Qualities

Restaurants and meat and poultry processing plants rely on chlorine bleach and other chlorine-based products to kill harmful levels of bacteria such as *Salmonella* and *E. coli* on food preparation surfaces and during food processing. Chlorine is so important in poultry processing that the US Department of Agriculture requires an almost constant chlorine rinse for much of the cutting equipment. In fact, no proven economical alternative to chlorine disinfection exists for use in meat and poultry processing facilities.

Properties

Because it is highly reactive, chlorine is usually found in nature bound with other elements like sodium, potassium, and magnesium. When chlorine is isolated as a free element, chlorine is a greenish yellow gas, which is 2.5 times heavier than air. It turns to a liquid state at -34°C (-29°F), and it becomes a yellowish crystalline solid at -103°C (-153°F). Chemists began experimenting with chlorine and chlorine compounds in the 18th century. They learned that chlorine has an extraordinary ability to extend a chemical bridge between various elements and compounds that would not otherwise react with each other. Chlorine has been especially useful in studying and synthesizing organic compounds -- compounds that have at least one atom of the element carbon in their molecular structure. All living organisms, including humans, are composed of organic compounds.

Chlorine is one of the most abundant chemical elements on Earth. It is ubiquitous in soils, minerals, plants and animals. Seawater is a huge reservoir of dissolved chlorine weathered from the continents and transported to the oceans by Earth's rivers.

Chlorine is also one of the most useful chemical elements. Each chemical element has its own set of unique properties and chlorine is known as a very reactive element--so reactive, in fact, that it is usually found combined with other elements in the form of compounds. More than 3,500 naturally occurring chlorinated organic (associated with living organisms) compounds alone have been identified.

Chlorine's chemical properties have been harnessed innovatively for good use. For example, this element plays a huge role in public health. Chlorine-based disinfectants are capable of removing a wide variety of disease-causing germs from drinking water and wastewater as well as from hospital and food production surfaces. Additionally, chlorine plays an important role in the manufacture of thousands of products we depend upon every day, including such diverse items as cars, computers, pharmaceuticals and military flak jackets. As the ninth largest chemical produced in the U.S. by volume, chlorine is truly a "workhorse chemical."

Released From the Salt of the Earth

Chlorine is produced industrially from the compound sodium chloride, one of the many salts found in geologic deposits formed from the slow evaporation of ancient seawater. When electricity is applied to a brine solution of sodium chloride, chlorine gas (Cl2), caustic soda (NaOH) and hydrogen gas (H_2) are generated according to the following reaction:

Salt	+	Water	→	Chlorine gas	+	Caustic soda	+	Hydrogen gas
$2\ NaCl$	+	$2\ H_2O$	→	Cl_2	+	$2\ NaOH$	+	H_2

Co-Products
As the reaction demonstrates, chlorine gas cannot be produced without producing caustic soda, so chlorine and caustic soda are known as "co-products," and their economics are inextricably linked. Caustic soda, also called "alkali," is used to produce a wide range of organic and inorganic chemicals and soaps. In addition, the pulp and paper, alumina and textiles industries use caustic soda in their manufacturing processes. Thus, the "chlor-alkali" industry obtains two very useful chemicals by applying electrical energy to sea salt.

Definitions

Chlorine Gas Feed Room
A chlorine gas feed room, for the purposes of this document, is a room that contains the chlorinator(s) and active cylinder(s) used to apply chlorine gas at a water or wastewater facility.

Chlorine Gas Storage Room
A chlorine gas storage room, for the purposes of this document, is a room other than a chlorine gas feed room, in which full, partial, or empty chlorine gas cylinders or ton containers are stored at a water or wastewater facility.

Gas Chlorinator
A gas chlorinator is a device used to meter and control the application rate of chlorine gas into a liquid. There is the danger of the gas escaping at a water or wastewater treatment facility. The gas chlorinator should be isolated from a water or wastewater treatment plant.

Chlorine Cabinet
A chlorine cabinet is a pre-assembled or factory built unit that contains the equipment used to apply chlorine gas at a water or wastewater treatment facility. It is isolated from a water or wastewater treatment plant.

Top photograph, a view of the top of a 150 gas cylinder. Bottom, always work in pairs when working around Chlorine. Here the hoist is being used to move the container.

Chemical Equations, Oxidation States, and Balancing of Equations

Before we breakdown chlorine and other chemicals, let's start with this review of basic chemical equations.

Beginning
The common chemical equation could be A + B --> C + D. This is chemical A + chemical B, the two reacting chemicals will go to products C + D, etc.

Oxidation
The term "oxidation" originally meant a reaction in which oxygen combines chemically with another substance, but its usage has long been broadened to include any reaction in which electrons are transferred.

Oxidation and reduction always occur simultaneously (redox reactions), and the substance which gains electrons is termed the oxidizing agent. For example, cupric ion is the oxidizing agent in the reaction: Fe (metal) + Cu++ --> Fe++ + Cu (metal); here, two electrons (negative charges) are transferred from the iron atom to the copper atom; thus the iron becomes positively charged (is oxidized) by loss of two electrons, while the copper receives the two electrons and becomes neutral (is reduced).

Electrons may also be displaced within the molecule without being completely transferred away from it. Such partial loss of electrons likewise constitutes oxidation in its broader sense and leads to the application of the term to a large number of processes, which at first sight might not be considered to be oxidation. Reaction of a hydrocarbon with a halogen, for example, CH_4 + 2 Cl --> CH_3Cl + HCl, involves partial oxidation of the methane; halogen addition to a double bond is regarded as an oxidation.

Dehydrogenation is also a form of oxidation; when two hydrogen atoms, each having one electron, are removed from a hydrogen-containing organic compound by a catalytic reaction with air or oxygen, as in oxidation of alcohol to aldehyde.

Oxidation Number
The number of electrons that must be added to or subtracted from an atom in a combined state to convert it to the elemental form; i.e., in barium chloride ($BaCl_2$) the oxidation number of barium is +2 and of chlorine is -1. Many elements can exist in more than one oxidation state.

Now, let us look at some common ions. An ion is the reactive state of the chemical, and is dependent on its place within the periodic table.

Have a look at the "periodic table of the elements". It is arranged in columns of elements, there are 18 columns. You can see column one, H, Li, Na, K, etc. These all become ions as H^+, Li^+, K^+, etc. The next column, column 2, Be, Mg, Ca etc. become ions Be^{2+}, Mg^{2+}, Ca^{2+}, etc. Column 18, He, Ne, Ar, Kr are inert gases. Column 17, F, Cl, Br, I, ionize to a negative F^-, Cl^-, Br^-, I^-, etc.

What you now need to do is memorize the table of common ions, both positive ions and negative ions.

Table of Common Ions

Positive Ions

Valency 1		Valency 2		Valency 3	
lithium	Li^+	magnesium	Mg_2^+	aluminum	Al_3^+
sodium	Na^+	calcium	Ca_2^+	iron III	Fe_3^+
potassium	K^+	strontium	Sr_2^+	chromium	Cr_3^+
silver	Ag^+	barium	Ba_2^+		
hydronium	H_3O^+	copper II	Cu_2^+		
(or hydrogen)	H^+	lead II	Pb_2^+		
ammonium	NH_4^+	zinc	Zn_2^+		
copper I	Cu^+	manganese II	Mn_2^+		
mercury I	Hg^+	iron II	Fe_2^+		
		tin II	Sn_2^+		

Negative Ions

Valency 1		Valency 2		Valency 3	
fluoride	F^-	oxide	O_2^-	phosphate	PO_4^{3-}
chloride	Cl^-	sulfide	S_2^-		
bromide	Br^-	carbonate	CO_3^{2-}		
iodide	I^-	sulfate	SO_4^{2-}		
hydroxide	OH^-	sulfite	SO_3^{2-}		
nitrate	NO_3^-	dichromate	$Cr_2O_7^-$		
bicarbonate	HCO_3^-	chromate	CrO_4^{2-}		
bisulphate	HSO_4^-	oxalate	$C_2O_4^{2-}$		
nitrite	NO_2^-	thiosulfate	$S_2O_3^{2-}$		
chlorate	ClO_3^-	tetrathionate	$S_4O_6^{2-}$		
permanganate	MnO_4^-	monohydrogen phosphate	HPO_4^{2-}		
hypochlorite	OCl^-				
dihydrogen phosphate	$H_2PO_4^-$				

Positive ions will react with negative ions, and vice versa. This is the start of our chemical reactions. For example:

$Na^+ + OH^- \rightarrow NaOH$ (sodium hydroxide)

$Na^+ + Cl^- \rightarrow NaCl$ (salt)

$3H^+ + PO_4^{3-} \rightarrow H_3PO_4$ (phosphoric acid)

$2Na^+ + S_2O_3^{2-} \rightarrow Na_2S_2O_3$

You will see from these examples, that if an ion of one (+), reacts with an ion of one (-) then the equation is balanced. However, an ion like PO_4^{3-} (phosphate) will require an ion of 3+ or an ion of one (+) (but needs three of these) to neutralize the 3- charge on the phosphate. So, what you are doing is balancing the charges (+) or (-) to make them zero, or cancel each other out.

For example, since aluminum exists in its ionic state as Al^{3+}, it will react with many negatively charged ions; for example: Cl^-, OH^-, SO_4^{2-}, PO_4^{3-}.

Let us do these examples and balance them.
$Al^{3+} + Cl^- \rightarrow AlCl$ **(incorrect)**
$Al^{3+} + 3Cl^- \rightarrow AlCl_3$ **(correct)**

How did we work this out?
Al^{3+} has three positives (3+)
Cl^- has one negative (-)
It will require **3 negative charges** to cancel out the **3 positive charges** on the aluminum (Al^{3+}).

When the **left hand side** of the equation is written, to balance the number of chlorine's (Cl^-) required, the number 3 is placed in front of the ion concerned, in this case Cl^-, becomes $3Cl^-$.

On the **right hand side** of the equation, where the ions have become a compound (a chemical compound), the number is transferred to after the relevant ion, Cl_3.

Another example:
$Al^{3+} + SO_4^{2-} \rightarrow AlSO_4$ (**incorrect**)
$2Al^{3+} + 3SO_4^{2-} \rightarrow Al_2(SO_4)_3$ (**correct**)

Let me give you an easy way of balancing:
Al is 3+
SO_4 is 2-
Simply transpose the number of positives (or negatives) for each ion, to the other ion, by placing this value of one ion, in front of the other ion. That is, Al^{3+} the 3 goes in front of the SO_4^{2-} as $3SO_4^{2-}$, and SO_4^{2-}, the 2 goes in front of the Al^{3+} to become $2Al^{3+}$. Then on the **right hand side** of the equation, this same number (now in front of each ion on the **left side** of the equation), is placed after each "ion" entity.

Let us again look at:
$Al^{3+} + SO_4^{2-} \rightarrow AlSO_4$ **(incorrect)**
$Al^{3+} + SO_4^{2-} \rightarrow Al_2(SO_4)_3$ **(correct)**
Put the three from the Al in front of the SO_4^{2-} and the 2 from the SO_4^{2-} in front of the Al^{3+}.
Equation becomes:
$2Al^{3+} + 3SO_4^{2-} \rightarrow Al_2(SO_4)_3$. You simply place the valency of one ion, as a whole number, in front of the other ion, and vice versa.

Remember to encase the SO_4 in brackets. **Why?** Because we are dealing with the sulfate ion, SO_4^{2-}, and it is this ion that is 2- charged (not just the O_4), so we have to ensure that the "ion" is bracketed. Now to check, the 2 times $3^+ = 6^+$, and 3 times $2^- = 6^-$. We have equal amounts of positive ions, and equal amounts of negative ions.

Another example:
NaOH + HCl --> ?
Na is Na^+, OH is OH^-, so this gave us NaOH. Originally, the one positive canceled the one negative.

HCl is $H^+ + Cl^-$, this gave us HCl.

Reaction is going to be the Na^+ reacting with a negatively charged ion. This will have to be the chlorine, Cl^-, because at the moment the Na^+ is tied to the OH^-. **So:** $Na^+ + Cl^-$ --> NaCl
The H+ from the HCl will react with a negative (-) ion this will be the OH^- from the NaOH.
So: $H^+ + OH^-$ --> H_2O (water).

The complete reaction can be written:
NaOH + HCl --> NaCl + H_2O. We have **equal amounts** of all atoms **each side** of the equation, so the equation is **balanced**.
or
$Na^+OH^- + H^+Cl^-$ --> $Na^+Cl^- + H^+OH^-$

Something More Difficult:
$Mg(OH)_2 + H_3PO_4$ --> ? (equation on left **not** balanced)
Mg^{2+} $2OH^- + 3H^+PO_4^{3-}$ --> ? (equation on left **not** balanced), so let us rewrite the equation in **ionic form**.

The Mg^{2+} **needs to react with a negatively charged ion**, this will be the PO_4^{3-},
so: $3Mg^{2+} + 2PO_4^{3-}$ --> $Mg_3(PO_4)_2$
(**Remember** the **swapping** of the positive or negative charges on the ions in the **left side** of the equation, and placing it in front of each ion, and then placing this number after each ion on the **right side** of the equation)

What is left is the H^+ from the H_3PO_4 and this will react with a negative ion, we only have the OH^- from the $Mg(OH)_2$ left for it to react with.
$6H^+ + 6OH^-$ --> $6H_2O$

Where did I get the 6 from? When I balanced the Mg^{2+} with the PO_4^{3-}, the equation became $3Mg^{2+} + 2PO_4^{3-}$ --> $Mg_3(PO_4)_2$

Therefore, I must have required $3Mg(OH)_2$ to begin with, and $2H_3PO_4$, (because we originally had $(OH)_2$ attached to the Mg, and H_3 attached to the PO_4. I therefore have $2H_3$ reacting with $3(OH)_2$. We have to write this, on the **left side** of the equation, as $6H^+ + 6OH^-$ because we need it in ionic form.
The equation becomes:
$6H^+ + 6OH^-$ --> $6H_2O$

The full equation is now balanced and is:
$3Mg(OH)_2 + 2H_3PO_4$ --> $Mg_3(PO_4)_2 + 6H_2O$
I have purposely split the equation into segments of reactions. This is showing you which ions are reacting with each other. Once you get the idea of equations you will not need this step.

The balancing of equations is simple. You need to learn the valency of the common ions (see tables). The rest is pure mathematics; you are balancing valency charges, positives versus

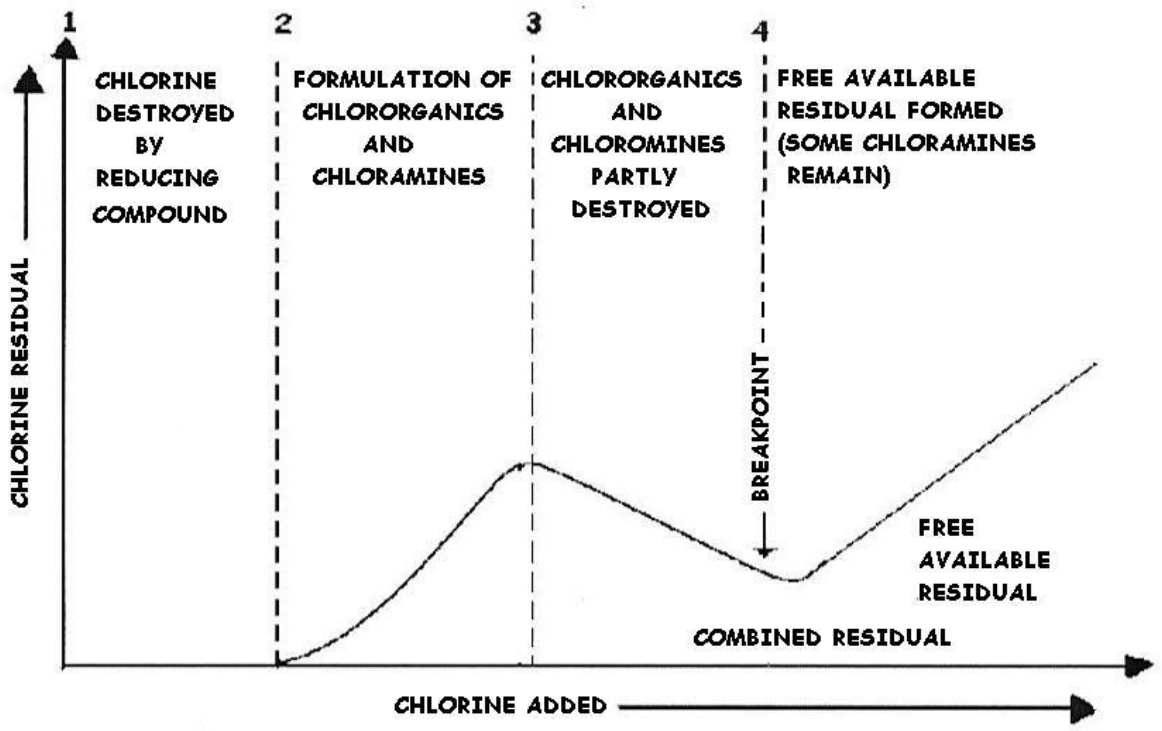
negatives. You have to have the **same number** of **negatives**, or **positives**, each side of the equation, and the **same number** of **ions** or **atoms** each side of the equation.

If one ion, example Al^{3+}, (3 positive charges) reacts with another ion, example OH^- (one negative ion) then we require 2 more negatively charged ions (in this case OH^-) to counteract the 3 positive charges the Al^{3+} contains.

Take my earlier hint, place the 3 from the Al^{3+} in front of the OH^-, now reads $3OH^-$, place the 1 from the hydroxyl OH^- in front of the Al^{3+}, now stays the same, Al^{3+} (the 1 is **never** written in chemistry equations).

$Al^{3+} + 3OH^- \rightarrow Al(OH)_3$

The 3 is simply written in front of the OH^-, a recognized ion, there are no brackets placed around the OH^-. On the right hand side of the equation, all numbers in front of each ion on the left hand side of the equation are placed after each same ion on the right side of the equation. Brackets are used in the right side of the equation because the result is a compound. Brackets are also used for compounds (reactants) in the left side of equations, as in $3Mg(OH)_2 + 2H_3PO_4 \rightarrow ?$



Hard to tell, but these are one ton chlorine gas containers. Notice the five gallon bucket of motor oil in the bottom photograph. Also notice that this photograph is the only eye wash station that we found during our inspection of 10 different facilities. Do you have an eye wash and emergency shower?

Chemistry of Chlorination

Chlorine can be added as sodium hypochlorite, calcium hypochlorite or chlorine gas. When any of these is added to water, chemical reactions occur as these equations show:

Cl 2 + H 2 O → HOCl + HCl
(chlorine gas) (water) (hypochlorous acid) (hydrochloric acid)

CaOCl + H 2 O → 2HOCl + Ca(OH)
(calcium hypochlorite) (water) (hypochlorous acid) (calcium hydroxide)

NaOCl + H 2 O → HOCl + Na(OH)
(sodium hypochlorite) (water) (hypochlorous acid) (sodium hydroxide)

All three forms of chlorine produce hypochlorous acid (HOCl) when added to water. Hypochlorous acid is a weak acid but a strong disinfecting agent. The amount of hypochlorous acid depends on the pH and temperature of the water. Under normal water conditions, hypochlorous acid will also chemically react and break down into a hypochlorite. ion

(OCl -): HOCl H + + OCl – Also expressed HOCl → H + + OCl –
(hypochlorous acid) (hydrogen) (hypochlorite ion)
The hypochlorite ion is a much weaker disinfecting agent than hypochlorous acid, about 100 times less effective.

Let's now look at how pH and temperature affect the ratio of hypochlorous acid to hypochlorite ions. As the temperature is decreased, the ratio of hypochlorous acid increases. Temperature plays a small part in the acid ratio. Although the ratio of hypochlorous acid is greater at lower temperatures, pathogenic organisms are actually harder to kill. All other things being equal, higher water temperatures and a lower pH are more conducive to chlorine disinfection.

Types of Residual

If water were pure, the measured amount of chlorine in the water should be the same as the amount added. But water is not 100% pure. There are always other substances (interfering agents) such as iron, manganese, turbidity, etc., which will combine chemically with the chlorine.

This is called the ***chlorine demand.*** Naturally, once chlorine molecules are combined with these interfering agents, they are not capable of disinfection. It is free chlorine that is much more effective as a disinfecting agent.

So let's look now at how free, total and combined chlorine are related. When a chlorine residual test is taken, either a total or a free chlorine residual can be read.

Total residual is all chlorine that is available for disinfection.

Total chlorine residual = free + combined chlorine residual.

Free chlorine residual is a much stronger disinfecting agent. Therefore, most water regulating agencies will require that your daily chlorine residual readings be of free chlorine residual.

Break-point chlorination is where the chlorine demand has been satisfied, and any additional chlorine will be considered ***free chlorine.***

Residual Concentration/Contact Time (CT) Requirements

Disinfection to eliminate fecal and coliform bacteria may not be sufficient to adequately reduce pathogens such as Giardia or viruses to desired levels. Use of the ***"CT"*** disinfection concept is recommended to demonstrate satisfactory treatment, since monitoring for very low levels of pathogens in treated water is analytically very difficult.

The CT concept, as developed by the United States Environmental Protection Agency (Federal Register, 40 CFR, Parts 141 and 142, June 29, 1989), uses the combination of disinfectant residual concentration (mg/L) and the effective disinfection contact time (in minutes) to measure effective pathogen reduction. The residual is measured at the end of the process, and the contact time used is the T10 of the process unit (time for 10% of the water to pass).

CT = Concentration (mg/L) x Time (minutes)

The effective reduction in pathogens can be calculated by reference to standard tables of required CTs.

Required Giardia/Virus Reduction
All surface water treatment systems shall ensure a minimum reduction in pathogen levels: 3-log reduction in Giardia; and 4-log reduction in viruses.

These requirements are based on unpolluted raw water sources with Giardia levels of = 1 cyst/100 L, and a finished water goal of 1 cyst/100,000 L (equivalent to 1 in 10,000 risk of infection per person per year). Higher raw water contamination levels may require greater removals as shown on Table 4.1.

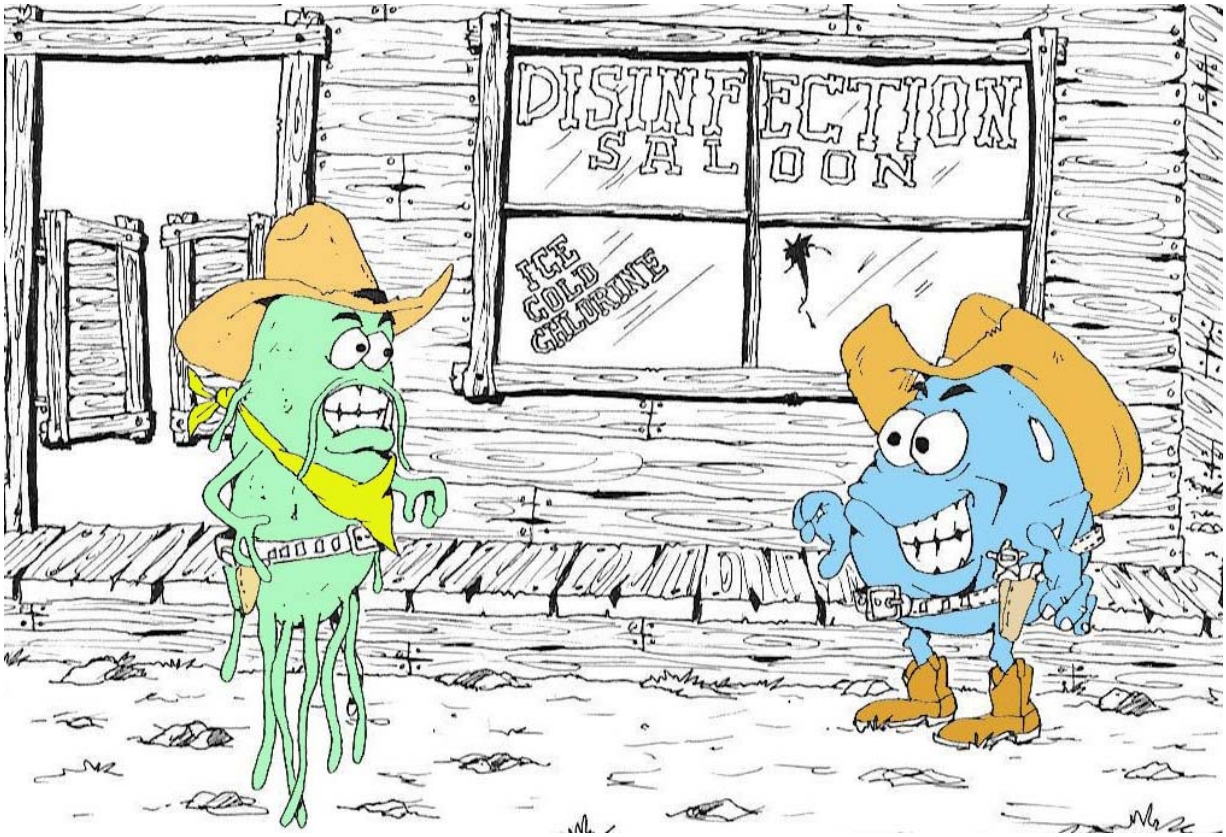
TABLE 4.1
Level of Giardia Reduction
Raw Water Giardia Levels*
Recommended Giardia Log Reduction
< 1 cyst/100 L 3-log
1 cyst/100 L - 10 cysts/100 L 3-log - 4-log
10 cysts/100 L - 100 cysts/100 L 4-log - 5-log
> 100 cysts/100 L > 5-log
*Use geometric means of data to determine raw water Giardia levels for compliance.

Required CT Value
Required CT values are dependent on pH, residual concentration, temperature, and the disinfectant used. The tables attached to Appendices A and B shall be used to determine the required CT.

Calculation and Reporting of CT Data
Disinfection CT values shall be calculated daily, using either the maximum hourly flow and the disinfectant residual at the same time, or by using the lowest CT value if it is calculated more frequently. Actual CT values are then compared to required CT values.

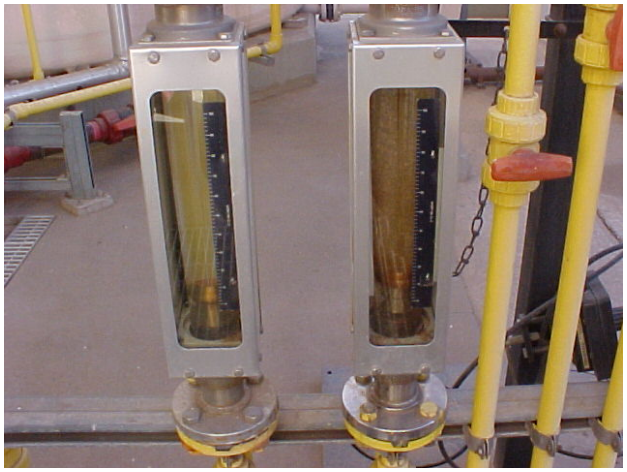
Results shall be reported as a reduction Ratio, along with the appropriate pH, temperature, and disinfectant residual. The reduction Ratio must be greater than 1.0 to be acceptable.

Users may also calculate and record actual log reductions.
Reduction Ratio = CT actual divide by CT required.



Chlorinator Components

A. **Ejector**
B. **Check Valve Assembly**
C. **Rate Valve**
D. **Diaphragm Assembly**
E. **Interconnection Manifold**
F. **Rotometer Tube and Float**
G. **Pressure Gauge**
H. **Gas Supply**



Chlorine measurement devices or Rotometers.

Chlorine Safety Information: There is a fusible plug on every chlorine tank. This metal plug will melt at 158 to 165° F. This is to prevent a build-up of excessive pressure and the possibility of cylinder rupture due to fire or high temperatures.

Chlorine Review

Chlorine Demand: The minimum amount of chlorine needed to react in a water purification system; used as a monitoring measurement by system operators.

Chlorine Residual: The concentration of chlorine in the water after the chlorine demand has been satisfied. The concentration is normally expressed in terms of total chlorine residual, which includes both the free and combined or chemically bound chlorine residuals.

Combined Chlorine Residual: The amount of chlorine used up in a water purification system; used as a monitoring measurement by system operators. Combined chlorine is defined as the residual chlorine existing in water in chemical combination with ammonia or organic amines which can be found in natural or polluted waters. Ammonia is sometimes deliberately added to chlorinated public water supplies to provide inorganic chloramines.

Free Chlorine: Free chlorine is defined as the concentration of residual chlorine in water present as dissolved gas (Cl_2), hypochlorous acid (HOCl), and/or hypochlorite ion (OCl-). The three forms of free chlorine exist together in equilibrium.

$$Cl_2 + H_2O \quad HOCl + H^+ + Cl^-$$

$$HOCl \quad H^+ + OCl^-$$

Their relative proportions are determined by the pH value and temperature. Regardless of whether pre-chloration is practiced or not, a free chlorine residual of at least 10 mg/L should be maintained in the clear well or distribution reservoir immediately downstream from the point of post-chlorination and .2 mg/L in the distribution system to guard against backflow.

Total Chlorine Residual: The total of free residual and combined residual chlorine in a water purification system; used as a monitoring measurement by system operators. Total chlorine is the sum of free and combined chlorine. When chlorinating most potable water supplies, total chlorine is essentially equal to free chlorine since the concentration of ammonia or organic nitrogen compounds (needed to form combined chlorine) will be very low. When chloramines are present in the municipal water supply, then total chlorine will be higher than free chlorine.

Pre-chlorination: The addition of chlorine at the plant headworks or prior to other water treatment or groundwater production processes and mainly used for disinfection and control of tastes, odors, and aquatic growths.

Post-chlorination: The addition of chlorine after a process or adding chlorine downstream to meet a demand in the system.

Breakpoint chlorination: Breakpoint chlorination means adding Cl_2 to the water until the Cl_2 demand is satisfied. Until all the microorganisms are killed.

What is the process of chlorination called as a treatment process and how does it differ from sterilization?
Chlorination: A method of water disinfection where gaseous, liquid, or dissolved chlorine is added to a water supply system. Water which has been treated with chlorine is effective in preventing the spread of disease. The chlorination of public drinking supplies was originally met with resistance, as people were concerned about the health effects of the practice. The use of chlorine has greatly reduced the prevalence of waterborne disease as it is effective against almost all bacteria and viruses, as well as amoeba. Sterilization kills everything.

What are the physical properties of chlorine, what hazards does it present, what advantages does it have over most other disinfectants, and how does it react with bacteria?
Physical and chemical properties of chlorine: A yellowish green, nonflammable and liquefied gas with an unpleasant and irritating smell. Can be readily compressed into a clear, amber-colored liquid, a noncombustible gas, and a strong oxidizer. Solid chlorine is about 1.5 times heavier than water and gaseous chlorine is about 2.5 times heavier than air. Atomic number of chlorine is 17. Cl is the elemental symbol and Cl2 is the chemical formula.

Chlorine reacts with bacteria as if it was very corrosive and burns the skin or covering killing the bacteria.

What is the purpose of a fusible plug, at what temperature does it melt, and where is it located on 150-lb. and 1-ton cylinders?
Fusible plug is a safety device that melts. If the temperature of a full Cl2 cylinder is increased by 50^{o} F or 30^{o} C, a rupture may occur. It will melt at 158 to 165 degrees F. It is found on the side of a 1 ton container and on top of the 150 pound cylinder and is located in the valve below the valve seat.

What is the correct procedure to follow in changing a chlorine cylinder and what item should always be replaced with a new one in doing so?
Hook up the chlorinator to the container or cylinder with the chlorine valve turned off. Use the gas side not the liquid if using a 1 ton container. Remove the cylinder valve outlet cap and check the valve face or damage. Clean with wire brush if necessary. If the valve face is smooth, clean proceed with hooking up the cylinder. Check the inlet face of the chlorinator and clean if necessary. Place a new lead gasket on the chlorinator inlet, place the chlorinator on the cylinder valve, install the yoke clamp and slowly tighten the yoke clamp until the two faces are against the lead gasket. Tighten the yoke, compressing the gasket one half to three quarters turn, do not over tighten. Replace the lead gasket with every change out.

How, when and where should chlorine residuals be taken and what information do they provide? The sample must be taken within the distribution system of your PWS. If you take it before the distribution system you will not get an accurate reading. The sample must be taken at the same tap that you take the Bac-t sample.

Chlor-Alkali Membrane Process

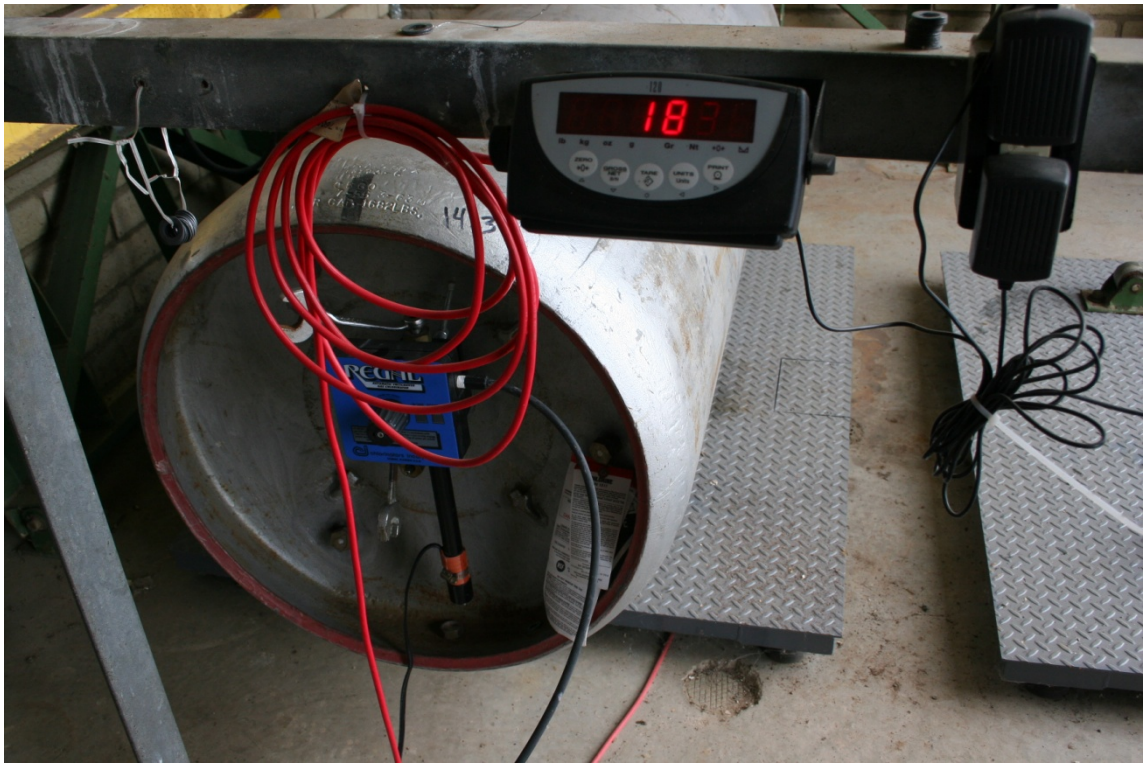
The electrolysis occurs in a cell containing electrodes submerged in solutions called electrolytes. One electrode is referred to as the anode and is submerged in a salt water solution. The second electrode is the cathode and is submerged in a sodium hydroxide (caustic soda) solution. A membrane is used to keep the two different solutions from mixing. This particular method of producing chlorine is called the chlor-alkali membrane process.

When a low voltage direct current (DC) power supply is applied to the electrodes in the cell, the sodium and chlorine ions in the brine are attracted in opposite directions to the polarized electrodes. The sodium ion passes across an ion selective membrane leaving the chlorine ion to combine with a second chlorine ion, which makes a chlorine gas bubble at the anode (electrode).

When the sodium crosses the membrane, it combines with a hydroxyl ion at the cathode (electrode) making sodium hydroxide, or caustic soda (NaOH). The hydroxyl ion originates from the dissolution of water at the cathode where hydrogen gas also develops. The membrane in the cell keeps the two solutions separate; otherwise, the chlorine gas bubble would immediately combine with the caustic soda forming sodium hypochlorite, or bleach. This process, which uses a membrane to separate the two solutions, is called the chlor-alkali process. The chemical equation for the chlor-alkali process is illustrated in the following equation:

$$2NaCl + 2H_2O + \text{electric current} \rightarrow (2NaOH + Cl_2 + H_2)$$

Top photograph, adjusting the Chlorine leak alarm sensor. Bottom photograph, Chlorine container weight scales.



Chlorine's Effectiveness

The effectiveness of chlorination depends on the chlorine demand of the water, the concentration of the chlorine solution added, the time that chlorine is in contact with the organism, and water quality. These effects can be summarized in the following manner:

- As the concentration of the chlorine increases, the required contact time to disinfect decreases.
- Chlorination is more effective as water temperature increases.
- Chlorination is less effective as the water's pH increases (becomes more alkaline).
- Chlorination is less effective in cloudy (turbid) water.
- When chlorine is added to the water supply, part of it combines with other chemicals in water (like iron, manganese, hydrogen sulfide, and ammonia) and is not available for disinfection. The amount of chlorine that reacts with the other chemicals plus the amount required to achieve disinfection is the **chlorine demand** of the water.

The safest way to be sure that the amount of chlorine added is sufficient is to add a little more than is required. This will result in a free chlorine residual that can be measured easily. This chlorine residual must be maintained for several minutes depending on chlorine level and water quality. Table 4 lists the free chlorine residual level needed for different contact times, water temperatures and pH levels.

Kits are available for measuring the chlorine residual by looking for a color change after the test chemical is added. The test is simple and easy for a homeowner to perform. If chlorination is required for the water supply, the chlorine residual should be tested regularly to make sure the system is working properly. The kit should specify that it measures the free chlorine residual and not the total chlorine. Once chlorine has combined with other chemicals it is not effective as a disinfectant. If a test kit does not distinguish between free chlorine and chlorine combined with other chemicals, the test may result in an overestimation of the chlorine residual.

Chlorine will kill bacteria in water, but it takes some time (Table 4) . The time needed depends on the concentration of chlorine. Two methods of chlorination are used to disinfect water: **simple chlorination** and **superchlorination**.

Table 4. Necessary chlorine residual to disinfect water for various contact times, water temperatures and pH			
Water Temp. 50 degrees F			
Contact time (minutes)	**Necessary chlorine residual (mg/l)**		
	pH 7	**pH 7.5**	**pH 8**
40	0.2	0.3	0.4
30	0.3	0.4	0.5
20	0.4	0.6	0.8
10	0.8	1.2	1.6
5	1.6	2.4	3.2
2	4.0	6.0	8.0
1	8.0	12.0	16.0
Water Temp. 32 - 40 degrees F			
Contact time (minutes)	**Necessary chlorine residual (mg/l)**		
	pH 7	**pH 7.5**	**pH 8**
40	0.3	0.5	0.6
30	0.4	0.6	0.8
20	0.6	0.9	1.2
10	1.2	1.8	2.4
5	2.4	3.6	4.8
2	6.0	9.0	12.0
1	12.0	18.0	24.0

Example: ***What is the necessary chlorine residual for well water with pH 7.5?***

The well water is 38 degrees F when it enters the house. The pump delivers 7 gallons per minute and after the chlorine is added it is held in a 100 gallon holding tank.

1. Contact time (from Table 5) - gallons per minute for 50 gallon tank = 5 minutes
2. Multiply by 2 for a 100 gallon tank = 10 minutes.
3. Necessary chlorine residual (from Table 4)- for water at 38 degrees F and pH 7.5 = **1.8 mg/l.**

Simple chlorination involves maintaining a low level of free residual chlorine at a concentration between 0.30 to .5 mg/l for at least 30 minutes. The residual is measured at the faucet most distant from the where chlorine is added to the water supply.

To ensure the proper contact time of at least 30 minutes, a holding tank can be installed (Table 5). Pressure tanks, while often thought to be sufficient, are usually too small to always provide 30 minutes of contact time.

Table 5. Available contact time from a 50-gallon holding tank	
Water flow rate (gallons per minute)	**Holding time (minutes)**
5	7
7	5
10	3.5

Another way to maintain necessary contact time is to run the chlorinated water through a coil of pipe (Table 6).

Table 6. Available contact time from 1000 feet of 1-1/4 inch pipe	
Water flow rate (gallons per minute)	**Holding time (minutes)**
5	9.2
7	6.6
10	4.6

When the water cannot be held for at least 30 minutes before it is used, super chlorination is an alternative. For **superchlorination**, a chlorine solution is added to the water to produce a chlorine residual of between 3.0 and 5.0 mg/l, which is about ten times stronger than for simple chlorination.

The necessary contact time for this concentration is reduced to less than five minutes (Table 4). The water will have a very strong chlorine smell. If this is not desirable, the chlorine can be removed just before it is used with a carbon filter (Note: may not be currently allowed under your Department of Health for private water supplies).

Oxidation Chemistry

Oxidation chemistry has long been an accepted and effective part of many water treatment programs. Oxidizing chemicals used in today's water treatment programs include: chlorine, chlorine dioxide, bromine, bromine/chlorine releasing compounds, ozone and hydrogen peroxide.

Oxidizing microbiocides are often found at the forefront of many cooling water treatment programs. In large volume or once-through cooling systems they are usually the primary biocide and often are the most cost-effective programs available to a plant. When selecting these economical and versatile chemicals, several factors should be considered before a technically sound program is implemented. Environmental and regulatory impact, system pH, process contamination, and equipment capital and maintenance expense all play a role in the decision-making process.

The primary killing mechanism these types of microbiocides use is oxidizing protein groups within a microorganism. Proteins are the basic components of essential cellular enzymes that are necessary for life-sustaining cellular processes such as respiration. The destruction of these proteins deprives the cell of its ability to carry out fundamental life functions and quickly kills it. One oxidant is chlorine dioxide, which appears to provide an additional killing mechanism. Chlorine dioxide is able to diffuse readily through hydrophobic lipid layers of an organism, allowing it to react with cellular amino acids, which directly inhibits protein synthesis. Since amino acids are the basic building blocks of all cellular proteins, destruction of these molecules has a devastating effect on the microorganism.

Staff shall be familiar with the locations of the chemical feed building as indicated by a posted site plan. Self-contained breathing apparatus (SCBA) and personal protective equipment should be facing the chemical feed building. Emergency repair kits “B” and “C” should be stored on site close to the chemical feed building.

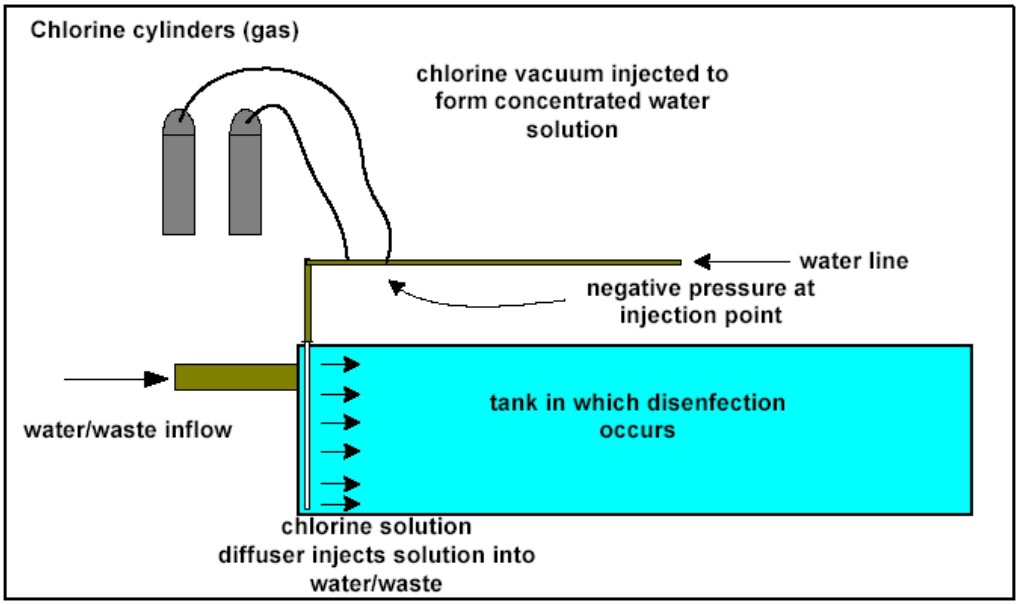
Chlorine scrubber

Chlorine Gas Section

Chlorine Gas

Background: Chlorine gas is a pulmonary irritant with intermediate water solubility that causes acute damage in the upper and lower respiratory tract. Chlorine gas was first used as a chemical weapon at Ypres, France in 1915. Of the 70,552 American soldiers poisoned with various gasses in World War I, 1843 were exposed to chlorine gas. Approximately 10.5 million tons and over 1 million containers of chlorine are shipped in the U.S. each year.

Chlorine is the most common chemical used to disenfect water and wastewater. It is added as a gas to most large installations. At very small facilities sodium hypochlorite (bleach) is added as a powder (tablets).



Chlorine is a yellowish-green gas at standard temperature and pressure. It is extremely reactive with most elements. Because its density is greater than that of air, the gas settles low to the ground. It is a respiratory irritant, and it burns the skin. Just a few breaths of it are fatal. Cl2 gas does not occur naturally, although Chlorine can be found in a number of compounds.

Chlorine gas is likely the most widely used oxidizing microbiocide. It has traditionally been the biocide of choice in many cooling water treatment systems. It is a strong oxidizer that is relatively easy to feed and is quite inexpensive. Upon introduction into the water stream, chlorine hydrolyzes into hypochlorous acid (HOCl) and hydrochloric acid (HCl).

This hydrolization provides the active toxicant, HOCl, which is pH-dependent. In alkaline cooling systems, it readily dissociates to form the hypochlorite ion (OCl-). This dissociation phenomenon is important to remember when working with systems that will operate at a higher pH. In alkaline conditions, OCl- becomes the predominant species and lacks the biocidal efficacy of the non-dissociated form. Considerably more HOCl is present at a pH of 7.0 than at pH 8.5.

It is also widely known that chlorine is non-selective, making it very sensitive to contamination from either cooling water makeup or from in-plant process leaks. Ammonia, organic acids and organic compounds, sulfides, iron and manganese all easily react with HOCl. The amount of chlorine needed to react with these contamination species is referred to as chlorine demand and it must be satisfied before active HOCl is available to provide a free chlorine residual.

The combination of high chlorine demand in process-contaminated systems and the dissociation process in alkaline systems creates the need for greater chlorine feed to obtain the same microbial efficacy. This results in a higher concentration of HCl in the cooling system. Since HCl removes alkalinity, pH depression and system corrosion could occur. In low pH water the passive metal oxide layers protecting the metal may resolubulize, exposing the surface to corrosion. At free mineral acidity (pH <4.3), many passivating inhibitors become ineffective, and corrosion will proceed rapidly. Increased chloride may also have a negative impact on system corrosion. The chloride ion (Cl^-) can damage or penetrate the passive oxide layer, leading to localized damage of the metal surface.

High chlorine concentrations have also been shown to directly attack traditional organic-based corrosion inhibitors. When these inhibitors are "deactivated," the metal surface would then be susceptible to corrosion. Process Safety Management (PSM) guidelines dictated by the U.S. Occupational Safety and Health Administration (OSHA), discharge problems related to chlorinated organic compounds such as trihalomethane (THM), dezincification of admiralty brass and delignification of cooling tower wood are other significant concerns associated with the use of chlorine.

Pathophysiology
Chlorine is a greenish-yellow, noncombustible gas at room temperature and atmospheric pressure. The intermediate water solubility of chlorine accounts for its effect on the upper airway and the lower respiratory tract.

Exposure to chlorine gas may be prolonged because its moderate water solubility may not cause upper airway symptoms for several minutes. In addition, the density of the gas is greater than that of air, causing it to remain near ground level and increasing exposure time.

The odor threshold for chlorine is approximately 0.3-0.5 parts per million (ppm); however, distinguishing toxic air levels from permissible air levels may be difficult until irritative symptoms are present.

Mechanism of Activity
The mechanisms of the above biological activity are poorly understood and the predominant anatomic site of injury may vary, depending on the chemical species produced. Cellular injury is believed to result from the oxidation of functional groups in cell components, from reactions with tissue water to form hypochlorous and hydrochloric acid, and from the generation of free oxygen radicals.

Although the idea that chlorine causes direct tissue damage by generating free oxygen radicals was once accepted, this idea is now controversial. The cylinders on the right contain chlorine gas.

The gas comes out of the cylinder through a gas regulator. The cylinders are on a scale that operators use to measure the amount used each day. The chains are used to prevent the tanks from falling over. Chlorine gas is stored in vented rooms that have panic bar equipped doors. Operators have the equipment necessary to reduce the impact of a gas leak, but rely on trained emergency response teams to contain leaks.

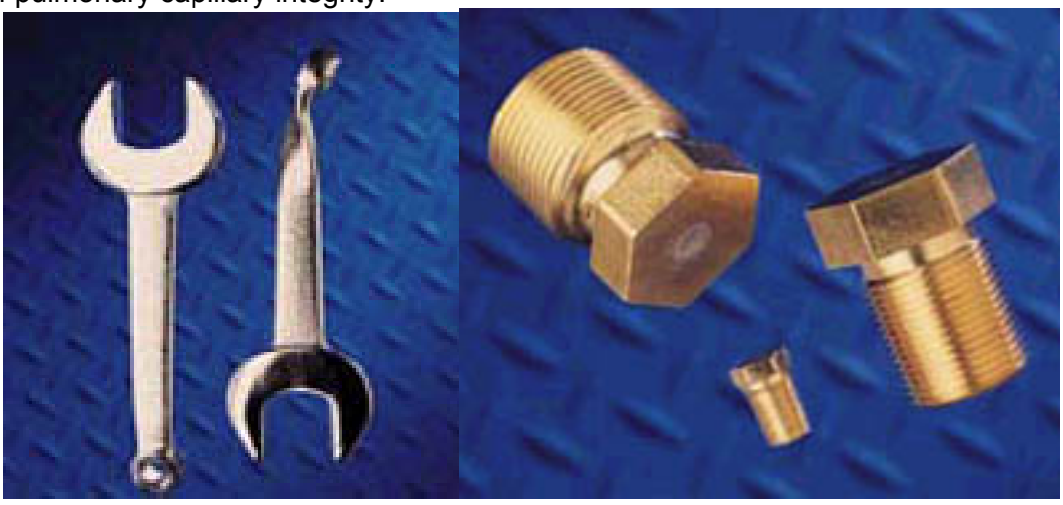
Solubility Effects
Hydrochloric acid is highly soluble in water. The predominant targets of the acid are the epithelia of the ocular conjunctivae and upper respiratory mucus membranes. Hypochlorous acid is also highly water soluble with an injury pattern similar to hydrochloric acid. Hypochlorous acid may account for the toxicity of elemental chlorine and hydrochloric acid to the human body.

Early Response to Chlorine Gas
Chlorine gas, when mixed with ammonia, reacts to form chloramine gas. In the presence of water, chloramines decompose to ammonia and hypochlorous acid or hydrochloric acid. The early response to chlorine exposure depends on the (1) concentration of chlorine gas, (2) duration of exposure, (3) water content of the tissues exposed, and (4) individual susceptibility.

Immediate Effects
The immediate effects of chlorine gas toxicity include acute inflammation of the conjunctivae, nose, pharynx, larynx, trachea, and bronchi. Irritation of the airway mucosa leads to local edema secondary to active arterial and capillary hyperemia. Plasma exudation results in filling the alveoli with edema fluid, resulting in pulmonary congestion.

Pathological Findings
Pathologic findings are nonspecific. They include severe pulmonary edema, pneumonia, hyaline membrane formation, multiple pulmonary thromboses, and ulcerative tracheobronchitis.

The hallmark of pulmonary injury associated with chlorine toxicity is pulmonary edema, manifested as hypoxia. Noncardiogenic pulmonary edema is thought to occur when there is a loss of pulmonary capillary integrity.



Chlorine Gas Cylinder Wrenches and Fusible Plugs.

Simple DPD Chlorine Field Test Kit.

Using DPD Method for Chlorine Residuals

N, N – diethyl-p-phenylenediame

Small portable chlorine measuring kit. The redder the mixture the "hotter" or stronger the chlorine in solution.

Measuring Chlorine Residual

Chlorine residual is the amount of chlorine remaining in water that can be used for disinfection. A convenient, simple and inexpensive way to measure chlorine residual is to use a small portable kit with pre-measured packets of chemicals that are added to water.

(Make sure you buy a test kit using the ***DPD method***, and not the outdated orthotolodine method.)

Chlorine test kits are very useful in adjusting the chlorine dose you apply. You can measure what chlorine levels are being found in your system (especially at the far ends).

Free chlorine residuals need to be checked and recorded daily. These results should be kept on file for a health or regulatory agency inspection during a regular field visit.

The most accurate method for determining chlorine residuals to use the laboratory amperometric titration method.

Amperometric Titration

The chlorination of water supplies and polluted waters serves primarily to destroy or deactivate disease-producing microorganisms. A secondary benefit, particularly in treating drinking water, is the overall improvement in water quality resulting from the reaction of chlorine with ammonia, iron, manganese, sulfide, and some organic substances.

Chlorination may produce adverse effects. Taste and odor characteristics of phenols and other organic compounds present in a water supply may be intensified. Potentially carcinogenic chloro-organic compounds such as chloroform may be formed.

Combined chlorine formed on chlorination of ammonia- or amine-bearing waters adversely affects some aquatic life. To fulfill the primary purpose of chlorination and to minimize any adverse effects, it is essential that proper testing procedures be used with a foreknowledge of the limitations of the analytical determination.

Chlorine applied to water in its molecular or hypochlorite form initially undergoes hydrolysis to form free chlorine consisting of aqueous molecular chlorine, hypochlorous acid, and hypochlorite ion. The relative proportion of these free chlorine forms is pH- and temperature-dependent. At the pH of most waters, hypochlorous acid and hypochlorite ion will predominate.

Free chlorine reacts readily with ammonia and certain nitrogenous compounds to form combined chlorine. With ammonia, chlorine reacts to form the chloramines: monochloramine, dichloramine, and nitrogen trichloride.

The presence and concentrations of these combined forms depend chiefly on pH, temperature, initial chlorine-to-nitrogen ratio, absolute chlorine demand, and reaction time. Both free and combined chlorine may be present simultaneously. Combined chlorine in water supplies may be formed in the treatment of raw waters containing ammonia or by the addition of ammonia or ammonium salts.

Chlorinated wastewater effluents, as well as certain chlorinated industrial effluents, normally contain only combined chlorine. Historically the principal analytical problem has been to distinguish between free and combined forms of chlorine.

Hach's AutoCAT 9000™ Automatic Titrator is the newest solution to hit the disinfection industry – a comprehensive, benchtop chlorine-measurement system that does it all: calibration, titration, calculation, real-time graphs, graphic print output, even electrode cleaning. More a laboratory assistant than an instrument, the AutoCAT 9000 gives you:

- High throughput, performs the titration and calculates concentration, all automatically.
- Forward titration, USEPA-accepted methods for free and total chlorine and chlorine dioxide with chlorite.
- Back titration, USEPA-accepted method for total chlorine in wastewater.
- Accurate, yet convenient: the easiest way to complete ppb-level amperometric titration.

Sodium Hypochlorite Section

Physical Properties - Sodium Hypochlorite
Description: Clear greenish yellow liquid.
Warning properties: Chlorine odor; inadequate warning of hazardous concentrations.
Molecular weight: 74.44 daltons
Boiling point (760 mm Hg): Decomposes above 40ºC (HSDB 2001)
Freezing point: 6ºC (21ºF)
Specific gravity: 1.21 (14% NAOCl solution) (water=1)
Water solubility: 29.3 g/100 g at 32ºF (0ºC)
Flammability: Not flammable

Alternative Names
Bleach; Clorox; Carrel-Dakin solution

Incompatibilities
Calcium or sodium hypochlorite react explosively or form explosive compounds with many common substances such as ammonia, amines, charcoal, or organic sulfides

Introduction
The world's most universal and reliable means of water and wastewater disinfection is chlorination. Two fundamental methods include gas chlorination (Cl2) and liquid chlorination (NaOCl) otherwise known as Sodium Hypochlorite. Sodium hypochlorite (NaOCl) is a solution made from reacting chlorine with a sodium hydroxide solution. These two reactants are the major co-products from most chlor-alkali cells. Sodium hypochlorite has a variety of uses and is an excellent disinfectant/antimicrobial agent. Sodium hypochlorite also significantly increases the pH of the water. When sodium hypochlorite is used, it must be counterbalanced by a strong acid like sodium bisulfate or muriatic acid to keep the pH within the ideal range.

The hypochlorite form of chlorine has been used since 1850. The most widely used form of hypochlorite is the liquid, sodium hypochlorite (NaOCl), with more than 150 tons per day consumed in the United States. Sodium hypochlorite application in cooling water is essentially the same as with gas chlorine; HOCl is produced as the active toxicant. The HOCl is equally susceptible to process contamination, has the same chlorine demand as gas chlorine and displays the same tendency to dissociate.

Sodium hypochlorite differs from chlorine gas in two respects: method of feed and hydrolization properties. Sodium hypochlorite can either be gravity-fed or applied with a metering pump. The latter is generally recognized as a consistently more accurate method. The second difference, in hydrolysis, lies in the end products. The NaOCl reaction with water liberates sodium hydroxide (NaOH).

The addition of NaOH differs in that it tends to add alkalinity to the water. In large concentrations it may artificially elevate pH, leading to precipitation of calcium carbonate. While NaOCl eliminates low pH corrosion as a concern, the use of large quantities in contaminated systems still introduces a high concentration of the chloride ion, which can be very aggressive to cooling system metals. Many of the other problems associated with chlorine remain present with sodium hypochlorite.

When was Sodium Hypochlorite Discovered?
Sodium hypochlorite has a long history. Around 1785 the Frenchman Berthollet developed liquid bleaching agents based on sodium hypochlorite. The Javel company introduced this product and called it 'liqueur de Javel'. At first, it was used to bleach cotton. Because of its specific characteristics it soon became a popular compound. Hypochlorite can remove stains from clothes at room temperature. In France, sodium hypochlorite is still known as 'eau de Javel'.

Characteristics of Sodium hypochlorite
Sodium hypochlorite is a clear, slightly yellowish solution with a characteristic odor.

Sodium hypochlorite has a relative density of is 1.1 (5.5% watery solution).

As a bleaching agent for domestic use it usually contains 5% sodium hypochlorite (with a pH of around 11, it is irritating). If it is more concentrated, it contains a concentration 10-15% sodium hypochlorite (with a pH of around 13, it burns and is corrosive).

Sodium hypochlorite is unstable. Chlorine evaporates at a rate of 0,75 gram active chlorine per day from the solution. Then heated sodium hypochlorite disintegrates. This also happens when sodium hypochlorite comes in contact with acids, sunlight, certain metals and poisonous and corrosive gasses, including chlorine gas. Sodium hypochlorite is a strong oxidator and reacts with flammable compounds and reductors. Sodium hypochlorite solution is a weak base that is inflammable. These characteristics must be kept in mind during transport, storage and use of sodium hypochlorite.

pH value When Sodium Hypochlorite is Added to Water
Due to the presence of caustic soda in sodium hypochlorite, the pH of the water is increased. When sodium hypochlorite dissolves in water, two substances form, which play a role in oxidation and disinfection. These are hypochlorous acid (HOCl) and the less active hypochlorite ion (OCl^-). The pH of the water determines how much hypochlorous acid is formed. While sodium hypochlorite is used, hydrochloric (HCl) is used to lower the pH. Sulfuric acid (H_2SO_4) can be used as an alternative for acetic acid. Less harmful gasses are produced when sulfuric acid is used. Sulfuric acid is a strong acid that strongly reacts with bases and is very corrosive.

How Can Sodium Hypochlorite be produced?
Sodium hypochlorite can be produced in two ways:
- By dissolving salt in softened water, which results in a concentrated brine solution. The solution is electrolyzed and forms a sodium hypochlorite solution in water. This solution contains 150 g active chlorine (Cl_2) per liter. During this reaction the explosive hydrogen gas is also formed.
- By adding chlorine gas (Cl_2) to caustic soda (NaOH). When this is done, sodium hypochlorite, water (H_2O) and salt (NaCl) are produced according to the following reaction:
$Cl_2 + 2NaOH \rightarrow NaOCl + NaCl + H_2O$

Applications of Sodium Hypochlorite
Sodium hypochlorite is used on a large scale; for example agriculture, chemical industries, paint- and lime industries, food industries, glass industries, paper industries, pharmaceutical industries, synthetics industries and waste disposal industries all use it. In the textile industry sodium hypochlorite is used to bleach textile. It is sometimes added to industrial waste water--this is done to reduce odors.

Hypochlorite neutralizes sulphur hydrogen gas (SH) and ammonia (NH3). It is also used to detoxify cyanide baths in metal industries. Hypochlorite can be used to prevent algae and shellfish growth in cooling towers. In water treatment, hypochlorite is used to disinfect water. In households, hypochlorite is used frequently for the purification and disinfection of the house.

How does Sodium Hypochlorite Disinfection Work?
By adding hypochlorite to water, hypochlorous acid (HOCl) is formed:
$NaOCl + H_2O \rightarrow HOCl + NaOH$

Hypochlorous acid is divided into hydrochloric acid (HCl) and oxygen (O). The oxygen atom is a very strong oxidant.

Sodium hypochlorite is effective against bacteria, viruses and fungi. Sodium hypochlorite disinfects the same way as chlorine does.

There are various ways to use sodium hypochlorite. For on-site salt electrolysis, a solution of salt (NaCl) in water is applied. Sodium (Na^+) and chloride (Cl^-) ions are produced.
$4NaCl \rightarrow 4Na^+ + 4Cl^-$

Subsequently, chlorine and hydroxide react to form hypochlorite:
$OH^- + Cl_2 \rightarrow HOCl + Cl^-$

You can work these problems in this section.

Salt Electrolysis System
The advantage of the salt electrolysis system is that no transport or storage of sodium hypochlorite is required. When sodium hypochlorite is stored for a long time, it becomes inactive. Another advantage of the onsite process is that chlorine lowers the pH and no other acid is required to lower pH.

The hydrogen gas that is produced is explosive and as a result ventilation is required for explosion prevention. This system is slow and a buffer of extra hypochlorous acid needs to be used. The maintenance and purchase of the electrolysis system is much more expensive than sodium hypochlorite.

When sodium hypochlorite is used, acetic or sulfuric acid are added to the water. An overdose can produce poisonous gasses. If the dosage is too low, the pH becomes too high and can irritate the eyes.

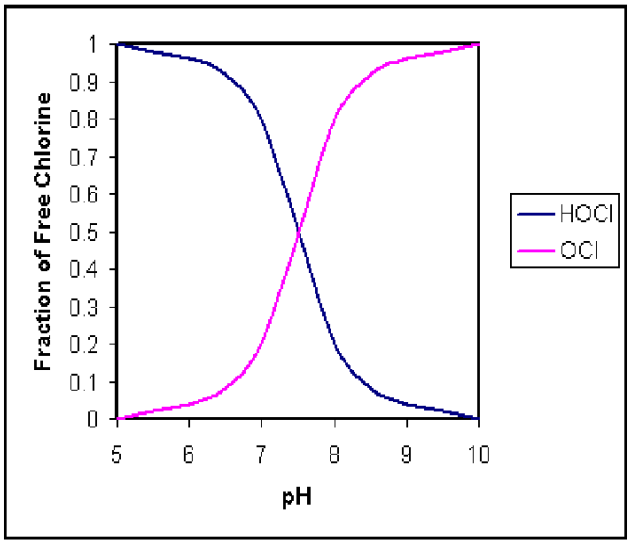
Because sodium hypochlorite is used both to oxidize pollutants (urine, sweat, cosmetics) and to remove pathogenic microorganisms, the required concentration of sodium hypochlorite depends on the concentrations of these pollutions. Especially the amount of organic pollutants helps determine the required concentration. If the water is filtered before sodium hypochlorite is applied, less sodium hypochlorite is needed.

Theory

Disinfection with chlorine is very popular in water and wastewater treatment because of its low cost, ability to form a residual, and its effectiveness at low concentrations. Although it is used as a disinfectant, it is a dangerous and potentially fatal chemical if used improperly.

Despite the fact the disinfection process may seem simple, it is actually a quite complicated process. Chlorination in wastewater treatment systems is a fairly complex science which requires knowledge of the plant's effluent characteristics.

When free chlorine is added to the wastewater, it takes on various forms depending on the pH of the wastewater. It is important to understand the forms of chlorine which are present because each has a different disinfecting capability. The acid form, HOCL, is a much stronger disinfectant than the hypochlorite ion, OCL-. The graph below depicts the chlorine fractions at different pH values (Drawing by Erik Johnston).



Ammonia present in the effluent can also cause problems as chloramines are formed, which have very little disinfecting power. Some methods to overcome the types of chlorine formed are to adjust the pH of the wastewater prior to chlorination or to simply add a larger amount of chlorine. An adjustment in the pH would allow the operators to form the most desired form of chlorine, hypochlorus acid, which has the greatest disinfecting power.

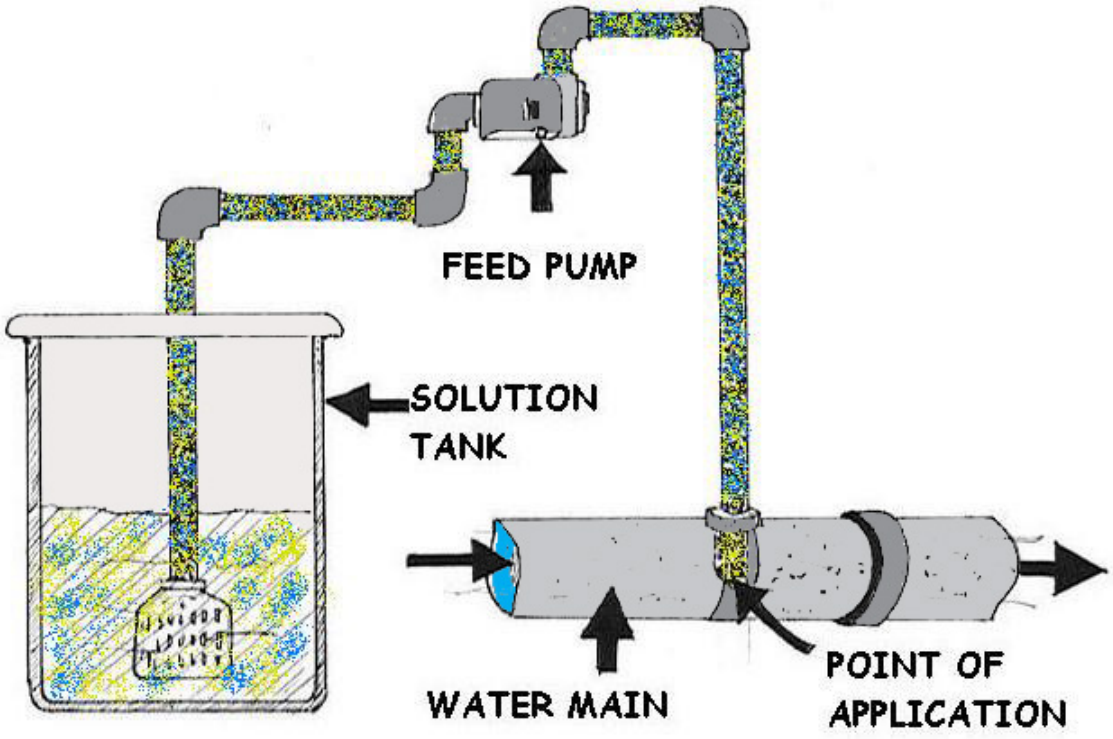
Adding larger amounts of chlorine would be an excellent method to combat the chloramines because the ammonia present would bond to the chlorine but further addition of chlorine would stay in the hypochlorus acid or hypochlorite ion state.

a) Chlorine gas, when exposed to water reacts readily to form hypochlorus acid, HOCl, and hydrochloric acid. Cl2 + H_2O -> HOCl + HCl

b) If the pH of the wastewater is greater than 8, the hypochlorus acid will dissociate to yield hypochlorite ion. HOCl <-> H^+ + OCl^-- If however, the pH is much less than 7, and then HOCl will not dissociate.

c) If ammonia is present in the wastewater effluent, then the hypochlorus acid will react to form one three types of chloramines depending on the pH, temperature, and reaction time.

Recommendations for Preparing/Handling/Feeding Sodium Hypochlorite Solutions



As a result of the pressures brought to bear by Health and Safety requirements, some users of gas have chosen to seek alternative forms of disinfectants for their water and wastewater treatment plants. One of these alternative forms is sodium hypochlorite (**NaOCl**)). This is often purchased commercially at 10 to 15% strength.

The handling and storage of NaOCl presents the plant with a new and sometimes unfamiliar, set of equipment installation configurations and operating conditions.

Product Stability The oxidizing nature of this substance means that it should be handled with extreme care. As NaOCl is relatively unstable, it degrades over time.

There are Three Ways in Which NaOCl Solutions Degrade

• Chlorate-forming reaction due to age, temperature, light and minor reduction in pH.
• Oxygen-producing reaction that occurs when metals, such as iron, copper or nickel, or metal oxides are brought into contact with the solution.
• Chlorine-producing reaction when solution pH falls below 6.

There are Many Factors that Affect the Stability of a NaOCl Solution

• Initial solution strength.
• pH solution.
• Temperature of the solution.
• Exposure of the solution to sunlight.

Exposure

There is no threshold value for to sodium hypochlorite exposure. Various health effects occur after exposure to sodium hypochlorite. People are exposed to sodium hypochlorite by inhalation of aerosols. This causes coughing and a sore throat. After swallowing sodium hypochlorite the effects are stomach ache, a burning sensation, coughing, diarrhea, a sore throat and vomiting. Sodium hypochlorite on skin or eyes causes redness and pain. After prolonged exposure, the skin can become sensitive. Sodium hypochlorite is poisonous for water organisms. It is mutagenic and very toxic when it comes in contact with ammonium salts.

Routes of Exposure
Inhalation
Hypochlorite solutions can liberate toxic gases such as chlorine. Chlorine's odor or irritant properties generally provide adequate warning of hazardous concentrations. However, prolonged, low-level exposures, such as those that occur in the workplace, can lead to olfactory fatigue and tolerance of chlorine's irritant effects. Chlorine is heavier than air and may cause asphyxiation in poorly ventilated, enclosed, or low-lying areas.

Children exposed to the same levels of gases as adults may receive a larger dose because they have greater lung surface area/body weight ratios and higher minute volumes/weight ratios. Children may be more vulnerable to corrosive agents than adults because of the smaller diameter of their airways. In addition, they may be exposed to higher levels than adults in the same location because of their short stature and the higher levels of chlorine found nearer to the ground.

Skin/Eye Contact
Direct contact with hypochlorite solutions, powder, or concentrated vapor causes severe chemical burns, leading to cell death and ulceration. Because of their relatively larger surface area/weight ratio, children are more vulnerable to toxicants affecting the skin.

Ingestion
Ingestion of hypochlorite solutions causes vomiting and corrosive injury to the gastrointestinal tract. Household bleaches (3 to 6% sodium hypochlorite) usually cause esophageal irritation, but rarely cause strictures or serious injury such as perforation. Commercial bleaches may contain higher concentrations of sodium hypochlorite and are more likely to cause serious injury. Metabolic acidosis is rare, but has been reported following the ingestion of household bleach. Pulmonary complications resulting from aspiration may also be seen after ingestion.

Sources/Uses
Sodium and calcium hypochlorite are manufactured by the chlorination of sodium hydroxide or lime. Sodium and calcium hypochlorite are used primarily as oxidizing and bleaching agents or disinfectants. They are components of commercial bleaches, cleaning solutions, and disinfectants for drinking water and waste water purification systems and swimming pools.

Sodium Hypochlorite as a Disinfectant has the Following Advantages:
It can be easily stored and transported when it is produced on-site. Dosage is simple; transport and storage of sodium hypochlorite are safe. Sodium hypochlorite is as effective as chlorine gas for disinfection. Sodium hypochlorite produces residual disinfectant.

Disadvantages
Sodium hypochlorite is a dangerous and corrosive substance. While working with sodium hypochlorite, safety measures have to be taken to protect workers and the environment.

Sodium hypochlorite should not come in contact with air, because that will cause it to disintegrate. Both sodium hypochlorite and chlorine do not deactivate Giardia Lambia and Cryptosporidium. The regulation for sodium hypochlorite is the same as the regulation considering chlorine.

Household bleaches usually contain sodium hypochlorite in a 3% to 6% solution. Some sodium hydroxide (lye) is added to keep the pH high to avoid decomposition. If the solution is made more acidic, sodium hypochlorite will dissociate, producing chlorine gas and oxygen. It is made by bubbling chlorine gas through a solution of sodium hydroxide. In the environment, it breaks down into water, oxygen, and table salt.

Conditions that tend to increase gassing in Sodium Hypochlorite Solutions are:
* Elevated temperatures
* High concentration solution
* Exposure to sunlight or UV rays
* Reduction in pressure
* Cavitation
* Poor piping conditions
* Contact with metallic impurities
* Contact with organic impurities
* Age of solution
* Quality of solution

Reciprocating Piston Metering Pumps
When handling sodium hypochlorite and acids, be certain to wear gloves and a face shield for protection. Sodium hypochlorite is introduced to treated water by a chemical feeder (pump.) Chemical feeders tend to clog often, so it's important to clean the feeder regularly. Sodium Hypochlorite is subject to degradation within the piping and pump systems as it releases oxygen gas and results in crystallization of the residual. If the oxygen gas or vapor is allowed to build up within the piping and reagent head in sufficient volume, a typical reciprocating piston metering pump, used for accurately feeding chlorine to the process, will not function properly as gas in the pump head is compressed, minimizing the discharge check valve to open upon discharge stroke of the pump. Consequently, this effect could require that the pump be re-primed for operation. Reciprocating piston metering pumps or diaphragm metering pumps have been historically preferred in the dispensing of Sodium Hypochlorite because of their superior ability to accurately dose chemicals into a process stream with great precision and repeatability at a constant pressure. Additionally, the diaphragm metering pump is sealless and leak proof by design with negligible maintenance and simple commissioning.

Traditionally, the diaphragm metering pump industry has promoted the use of degas valves on the discharge port of the pump which diverts gas back to the suction supply source of the bleach. This method has been widely accepted and successful in many applications. However, the small diameter ports in the valve system tend to plug and require continuous flushing or cleaning through human intervention since the system is open to atmosphere on the discharge side of the orifice. Additionally, an external bypass piping system and degas valve assembly require additional costs and maintenance while presenting more opportunities for undesired chlorine leak paths.

A ten minute emergency air escape pack. We suggest that any one that utilizes chlorine gas purchase several of these type devices and station these around the facility and in your work vehicle.

Troubleshooting Hypochlorination Problems for Effluent

Problem

1. Chemical feed pump won't run.
2. Low chlorine residual at POE.
2. Low chlorine residual at POE.
3. Chemical feed pump won't prime.
4. Loss of prime

Possible Causes

1A. No power.
1B. Electrical problem with signal from well pump or flow sensor.
1C. Motor failure.
2A. Improper procedure for running chlorine residual test or expired chemical reagents.
2B. Pump not feeding an adequate quantity of chlorine.
2C. Change in raw water quality.
2D. Pump **air bound**.
2E. Chlorine supply tank empty.
2F. Reduced effectiveness of chlorine solution.
2G. Damaged suction or discharge lines. (cracks or crimps)
2H. Connection at point of **injection** clogged or leaking.
3A. Speed and stroke setting inadequate.
3B. Suction lift too high due to feed pump relocation.
3C. Discharge pressure too high.
3D. Suction fitting clogged.
3E. Trapped air in suction line.
3F. Suction line not submerged in solution.
4A. Solution tank empty.
4B. Air leaks in suction fittings.
4C. Foot valve not in vertical position.
4D. Air trapped in suction tubing.

Possible Solutions

1A. Check to see if plug is securely in place.
Insure that there is power to the outlet and control systems.
1B. Check pump motor starter. Bypass flow sensor to determine if pump will operate manually.
1C. Check manufacturer's information.
2A Check expiration date on **chemical reagents**. Check test procedure as described in test kit manual. Speed or stroke setting too low.
2B. Damaged **diaphragm** or suction leak.
2C. Test raw water for constituents that may cause increased chlorine demand. (i.e. iron, manganese, etc.)
2D. Check foot valve.
2E. Fill supply tank.
2F. Check date that chlorine was received. Sodium hypochlorite solution may lose effectiveness after 30 days. If that is the case, the feed rate must be increased to obtain the desired residual.
2G. Clean or repair lines with problems.

2H. Flush line and connection with mild acid such as **Acetic** or **Muriatic**. Replace any damaged parts that may be leaking.
3A. Check manufacturers' recommendations for proper settings to prime pump.
3B. Check maximum suction lift for pump and relocate as necessary.
3C. Check well pump discharge pressure.
Check pressure rating on chemical feed pump.
3D. Clean or replace screen.
3E. Insure all fittings are tight.
3F. Add chlorine solution to supply tank.
4A. Fill tank.
4B. Check for cracked fittings.
4C. Adjust foot valve to proper position.
4D. Check connections and fittings.

Small Chlorine solution tank.

Risks and Benefits of Chlorine

Current evidence indicates that the benefits of chlorinating our wastewater include reduced incidence of water-borne diseases. Although other disinfectants are available, chlorine continues to be the choice of wastewater treatment experts. When used with primary and secondary treatment practices, chlorine is effective against bacteria, viruses and protozoa. It is easy to apply, and, most importantly, small amounts of chlorine remain in the water and continue to

disinfect. This ensures that the water remains free of microbial contamination on its journey from the wastewater treatment plant to the final outfall.

The risk of using chlorine is due to storage and application. Chlorine is considered hazardous material and proper training is very crucial. The photographs on this page show how not to store, secure, and operate chlorine containers.

These two photographs show easy access without any security. Terrorists would love to find this facility. Believe it or not, we have found several unprotected Cl_2 storage sites.

Notice the containers and see that these are not secured from rolling.

Health Hazard Information

Routes of Exposure
Exposure to chlorine can occur through inhalation, ingestion, and eye or skin contact [Genium 1992].

Summary of toxicology
1. Effects on Animals: Chlorine is a severe irritant of the eyes, mucous membranes, skin, and lungs in experimental animals. The 1 hour LC(50) is 239 ppm in rats and 137 ppm in mice ()[Sax and Lewis 1989]. Animals surviving sub-lethal inhalation exposures for 15 to 193 days showed marked emphysema, which was associated with bronchiolitis and pneumonia [Clayton and Clayton 1982]. Chlorine injected into the anterior chamber of rabbits' eyes resulted in severe damage with inflammation, opacification of the cornea, atrophy of the iris, and injury to the lens [Grant 1986].

2. Effects on Humans: Severe acute effects of chlorine exposure in humans have been well documented since World War I when chlorine gas was used as a chemical warfare agent. Other severe exposures have resulted from the accidental rupture of chlorine tanks. These exposures have caused death, lung congestion, and pulmonary edema, pneumonia, pleurisy, and bronchitis [Hathaway et al. 1991]. The lowest lethal concentration reported is 430 ppm for 30 minutes [Clayton and Clayton 1982].

Exposure to 15 ppm causes throat irritation, exposures to 50 ppm are dangerous, and exposures to 1000 ppm can be fatal, even if exposure is brief [Sax and Lewis 1989; Clayton and Clayton 1982]. Earlier literature reported that exposure to a concentration of about 5 ppm caused respiratory complaints, corrosion of the teeth, inflammation of the mucous membranes of the nose and susceptibility to tuberculosis among chronically-exposed workers.

However, many of these effects are not confirmed in recent studies and are of very dubious significance [ACGIH 1991]. A study of workers exposed to chlorine for an average of 10.9 years was published in 1970. All but six workers had exposures below 1 ppm; 21 had TWAs above 0.52 ppm. No evidence of permanent lung damage was found, but 9.4 percent had abnormal EKGs compared to 8.2 percent in the control group.

The incidence of fatigue was greater among those exposed above 0.5 ppm [ACGIH 1991]. In 1981, a study was published involving 29 subjects exposed to chlorine concentrations up to 2.0 ppm for 4- and 8-hour periods. Exposures of 1.0 ppm for 8 hours produced statistically significant changes in pulmonary function that were not observed at a 0.5 ppm exposure concentration. Six of 14 subjects exposed to 1.0 ppm for 8 hours showed increased mucous secretions from the nose and in the hypopharynx.

Responses for sensations of itching or burning of the nose and eyes, and general discomfort were not severe, but were perceptible, especially at the 1.0 ppm exposure level [ACGIH 1991]. A 1983 study of pulmonary function at low concentrations of chlorine exposure also found transient decreases in pulmonary function at the 1.0 ppm exposure level, but not at the 0.5 ppm level [ACGIH 1991]. Acne (chloracne) is not unusual among persons exposed to low concentrations of chlorine for long periods of time. Tooth enamel damage may also occur [Parmeggiani 1983]. There has been one confirmed case of myasthenia gravis associated with chlorine exposure [NLM 1995].

Signs and Symptoms of Exposure

1. Acute exposure: Acute exposure to low levels of chlorine results in eye, nose, and throat irritation, sneezing, excessive salivation, general excitement, and restlessness. Higher concentrations causes difficulty in breathing, violent coughing, nausea, vomiting, cyanosis, dizziness, headache, choking, laryngeal edema, acute tracheobronchitis, chemical pneumonia. Contact with the liquid can result in frostbite burns of the skin and eyes [Genium 1992].
2. Chronic exposure: Chronic exposure to low levels of chlorine gas can result in a dermatitis known as chloracne, tooth enamel corrosion, coughing, severe chest pain, sore throat, hemoptysis and increased susceptibility to tuberculosis [Genium 1992].

Emergency Medical Procedures: [NIOSH to supply]

1. Rescue: Remove an incapacitated worker from further exposure and implement appropriate emergency procedures (e.g., those listed on the Material Safety Data Sheet required by OSHA's Hazard Communication Standard [29 CFR 1910.1200]).
2. All workers should be familiar with emergency procedures, the location and proper use of emergency equipment, and methods of protecting themselves during rescue operations.

Exposure Sources and Control Methods

The following operations may involve chlorine and lead to worker exposures to this substance:

The Manufacture and Transportation of Chlorine

- Use as a chlorinating and oxidizing agent in organic and inorganic synthesis; in the manufacture of chlorinated solvents, automotive antifreeze and antiknock compounds, polymers (synthetic rubber and plastics), resins, elastomers, pesticides, refrigerants, and in the manufacture of rocket fuel.
- Use as a fluxing, purification, and extraction agent in metallurgy.
- Use as a bacteriostat, disinfectant, odor control, and demulsifier in treatment of drinking water, swimming pools, and in sewage.
- Use in the paper and pulp, and textile industries for bleaching cellulose for artificial fibers; use in the manufacture of chlorinated lime; use in de-tinning and de-zincing iron; use to shrink-proof wool.
- Use in the manufacture of pharmaceuticals, cosmetics, lubricants, flame-proofing, adhesives, in special batteries containing lithium or zinc, and in hydraulic fluids; use in the processing of meat, fish, vegetables, and fruit.
- Use as bleaching and cleaning agents, and as a disinfectant in laundries, dishwashers, cleaning powders, cleaning dairy equipment, and bleaching cellulose.

Methods that are effective in controlling worker exposures to chlorine, depending on the feasibility of implementation, are as follows: Process enclosure Local exhaust ventilation General dilution ventilation Personal protective equipment.

Workers responding to a release or potential release of a hazardous substance must be protected as required by paragraph (q) of OSHA's Hazardous Waste Operations and Emergency Response Standard 29 CFR.

Good Sources of Information about Control Methods are as Follows:

1. ACGIH [1992]. Industrial ventilation--a manual of recommended practice. 21st ed. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.
2. Burton DJ [1986]. Industrial ventilation--a self-study companion. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.
3. Alden JL, Kane JM [1982]. Design of industrial ventilation systems. New York, NY: Industrial Press, Inc.
4. Wadden RA, Scheff PA [1987]. Engineering design for control of workplace hazards. New York, NY: McGraw-Hill.
5. Plog BA [1988]. Fundamentals of industrial hygiene. Chicago, IL: National Safety Council.

Chlorine Storage

Chlorine should be stored in a cool, dry, well-ventilated area in tightly sealed containers that are labeled in accordance with OSHA's Hazard Communication Standard [29 CFR 1910.1200]. Containers of chlorine should be protected from exposure to weather, extreme temperatures changes, and physical damage, and they should be stored separately from flammable gases and vapors, combustible substances (such as gasoline and petroleum products, hydrocarbons, turpentine, alcohols, acetylene, hydrogen, ammonia, and sulfur), reducing agents, finely divided metals, arsenic, bismuth, boron, calcium, activated carbon, carbon disulfide, glycerol, hydrazine, iodine, methane, oxomonosilane, potassium, propylene, silicon, hydrogen sulfide and water, carbon monoxide and sulfur dioxide, moisture, steam, and water. (Sulfur dioxide is used for de-chlorination).

Workers handling and operating chlorine containers, cylinders, and tank wagons should receive special training in standard safety procedures for handling compressed corrosive gases. All pipes and containment used for chlorine service should be regularly inspected and tested. Empty containers of chlorine should have secured protective covers on their valves and should be handled appropriately.

Spills and Leaks

In the event of a spill or leak involving chlorine, persons not wearing protective equipment and fully-encapsulating, vapor-protective clothing should be restricted from contaminated areas until cleanup has been completed. The following steps should be undertaken following a spill or leak:

1. Notify safety personnel.
2. Remove all sources of heat and ignition.
3. Keep all combustibles (wood, paper, oil, etc.) away from the leak.
4. Ventilate potentially explosive atmospheres.
5. Evacuate the spill area for at least 50 feet in all directions.
6. Find and stop the leak if this can be done without risk; if not, move the leaking container to an isolated area until gas has dispersed. The cylinder may be allowed to empty through a reducing agent such as sodium bisulfide and sodium bicarbonate.
7. Use water spray to reduce vapors; do not put water directly on the leak or spill area.

Special Requirements

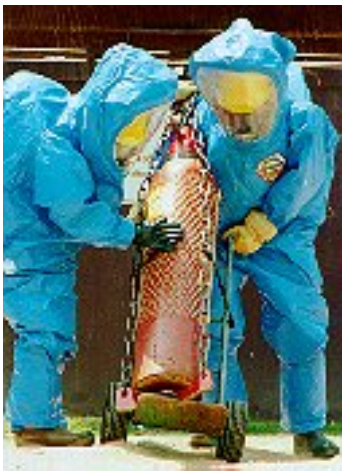
The U.S. Environmental Protection Agency (**EPA**) requirements for emergency planning, reportable quantities of hazardous releases, community right-to-know, and hazardous waste management may change over time. Users are therefore advised to determine periodically whether new information is available.

Emergency Planning Requirements
Employers owning or operating a facility at which there are 100 pounds or more of chlorine must comply with the EPA's emergency planning requirements [40 CFR Part 355.30].

Reportable Quantity Requirements for Hazardous Releases
A hazardous substance release is defined by the EPA as any spilling, leaking, pumping, pouring, emitting, emptying, discharging, injecting, escaping, leaching, dumping, or disposing into the environment including the abandonment or discarding of contaminated containers) of hazardous substances. In the event of a release that is above the reportable quantity for that chemical, employers are required to notify the proper Federal, State, and local authorities [40 CFR

The Reportable Quantity of Chlorine is 10 Pounds.
If an amount equal to or greater than this quantity is released within a 24-hour period in a manner that will expose persons outside the facility, employers are required to do the following: Notify the National Response Center immediately at (800) or at (202) 426-2675 in Washington, D.C. [40 CFR 302.6]. Notify the emergency response commission of the State likely to be affected by the release [40 CFR 355.40]. Notify the community emergency coordinator of the local emergency planning committee (or relevant local emergency response personnel) of any area likely to be affected by the release [40 CFR 355.40].

Community Right-to-Know Requirements
Employers who own or operate facilities in SIC codes 20 to 39 that employ 10 or more workers and that manufacture 25,000 pounds or more of chlorine per calendar year or otherwise use 10,000 pounds or more of chlorine per calendar year are required by EPA [40 CFR Part 372.30] to submit a Toxic Chemical Release Inventory form (Form R) to the EPA reporting the amount of chlorine emitted or released from their facility annually.



Hazardous Waste Management Requirements
EPA considers a waste to be hazardous if it exhibits any of the following characteristics: ignitability, corrosivity, reactivity, or toxicity as defined in 40 CFR 261.21-261.24. Under the Resource Conservation and Recovery Act (**RCRA**) [40 USC 6901 et seq.], the EPA has specifically listed many chemical wastes as hazardous. Although chlorine is not specifically listed as a hazardous waste under RCRA, the EPA requires employers to treat waste as hazardous if it exhibits any of the characteristics discussed above.

Providing detailed information about the removal and disposal of specific chemicals is beyond the scope of this guideline. The U.S. Department of Transportation, the EPA, and State and local regulations should be followed to ensure that removal, transport, and disposal of this substance are conducted in accordance with existing regulations.

Using DPD Method for Chlorine Residuals

Small portable chlorine measuring kit. The redder the mixture the "hotter" or stronger the chlorine in solution.

Measuring Chlorine Residual
Chlorine residual is the amount of chlorine remaining in water that can be used for disinfection. A convenient, simple and inexpensive way to measure chlorine residual is to use a small portable kit with pre-measured packets of chemicals that are added to water.

(Make sure you buy a test kit using the ***DPD method***, and not the outdated orthotolodine method.)

Chlorine test kits are very useful in adjusting the chlorine dose you apply. You can measure what chlorine levels are being found in your system (especially at the far ends).

Free chlorine residuals need to be checked and recorded daily. These results should be kept on file for a health or regulatory agency inspection during a regular field visit.

The most accurate method for determining chlorine residuals is to use the laboratory amperometric titration method.

Chlorination Equipment Requirements

For all wastewater treatment facilities, chlorine gas under pressure shall not be permitted outside the chlorine room. A chlorine room is where chlorine gas cylinders and/or ton containers are stored. Vacuum regulators shall also be located inside the chlorine room. The chlorinator, which is the mechanical gas proportioning equipment, may or may not be located inside the chlorine room.

For new and upgraded facilities, from the chlorine room, chlorine gas vacuum lines should be run as close to the point of solution application as possible. Injectors should be located to minimize the length of pressurized chlorine solution lines. A gas pressure relief system shall be included in the gas vacuum line between the vacuum regulator(s) and the chlorinator(s) to ensure that pressurized chlorine gas does not enter the gas vacuum lines leaving the chlorine room.

The gas pressure relief system shall vent pressurized gas to the atmosphere at a location that is not hazardous to plant personnel; vent line should be run in such a manner that moisture collecting traps are avoided. The vacuum regulating valve(s) shall have positive shutdown in the event of a break in the downstream vacuum lines.

As an alternative to chlorine gas, it is permissible to use hypochlorite with positive displacement pumping. Anti-siphon valves shall be incorporated in the pump heads or in the discharge piping.

Capacity
The chlorinator shall have the capacity to dose enough chlorine to overcome the demand and maintain the required concentration of the "***free***" or "***combined***" chlorine.

Methods of Control
Chlorine feed system shall be automatic proportional controlled, automatic residual controlled, or compound loop controlled. In the automatic proportional controlled system, the equipment adjusts the chlorine feed rate automatically in accordance with the flow changes to provide a constant pre-established dosage for all rates of flow. In the automatic residual controlled system, the chlorine feeder is used in conjunction with a chlorine residual analyzer which controls the feed rate of the chlorine feeders to maintain a particular residual in the treated water.

In the compound loop control system, the feed rate of the chlorinator is controlled by a flow proportional signal and a residual analyzer signal to maintain particular chlorine residual in the water.

A manual chlorine feed system may be installed for groundwater systems with constant flow rates.

Standby Provision
As a safeguard against malfunction and/or shut-down, standby chlorination equipment having the capacity to replace the largest unit shall be provided. For uninterrupted chlorination, gas chlorinators shall be equipped with an automatic changeover system. In addition, spare parts shall be available for all chlorinators.

Weigh Scales
Scales for weighing cylinders shall be provided at all plants using chlorine gas to permit an accurate reading of total daily weight of chlorine used. At large plants, scales of the recording and indicating type are recommended. As a minimum, a platform scale shall be provided. Scales shall be of corrosion-resistant material.

Securing Cylinders
All chlorine cylinders shall be securely positioned to safeguard against movement. Tag the cylinder *"**empty**"* and store upright and chained.

Ton containers may not be stacked.

Chlorine Leak Detection
Automatic chlorine leak detection and related alarm equipment shall be installed at all water treatment plants using chlorine gas. Leak detection shall be provided for the chlorine rooms. Chlorine leak detection equipment should be connected to a remote audible and visual alarm system and checked on a regular basis to verify proper operation.

Leak detection equipment shall not automatically activate the chlorine room ventilation system in such a manner as to discharge chlorine gas. During an emergency, if the chlorine room is unoccupied, the chlorine gas leakage shall be contained within the chlorine room itself in order to facilitate a proper method of clean-up.

Consideration should also be given to the provision of caustic soda solution reaction tanks for absorbing the contents of leaking one-ton cylinders where such cylinders are in use.

Chlorine leak detection equipment may not be required for very small chlorine rooms with an exterior door (e.g., floor area less than $3m^2$).

You can use a spray solution of Ammonia or a rag soaked with Ammonia to detect a small Cl_2 leak. If there is a leak, the ammonia will create a white colored smoke, Ammonium Chloride.

Safety Equipment
The facility shall be provided with personnel safety equipment including the following: Respiratory equipment; safety shower, eyewash; gloves; eye protection; protective clothing; cylinder and/or ton repair kits.

Respiratory equipment shall be provided which has been approved under the Occupational Health and Safety Act, General Safety Regulation - Selection of Respiratory Protective Equipment. Equipment shall be in close proximity to the access door(s) of the chlorine room.

Chlorine Room Design Requirements
Where gas chlorination is practiced, the gas cylinders and/or the ton containers up to the vacuum regulators shall be housed in a gas-tight, well illuminated, corrosion resistant and mechanically ventilated enclosure. The chlorinator may or may not be located inside the chlorine room. The chlorine room shall be located at the ground floor level.

Ventilation
Gas chlorine rooms shall have entirely separate exhaust ventilation systems capable of delivering one (1) complete air change per minute during periods of chlorine room occupancy only. The air outlet from the room shall be 150 mm above the floor and the point of discharge located to preclude contamination of air inlets to buildings or areas used by people. The vents to the outside shall have insect screens.

Air inlets should be louvered near the ceiling, the air being of such temperature as to not adversely affect the chlorination equipment. Separate switches for fans and lights shall be outside the room at all entrance or viewing points, and a clear wire-reinforced glass window shall be installed in such a manner as to allow the operator to inspect from the outside of the room.

Heating
Chlorine rooms shall have separate heating systems, if a forced air system is used to heat the building. The hot water heating system for the building will negate the need for a separate heating system for the chlorine room. The heat should be controlled at approximately 15^{o}C.

Cylinders or containers shall be protected to ensure that the chlorine maintains its gaseous state when entering the chlorinator.

Access
All access to the chlorine room shall only be from the exterior of the building. Visual inspection of the chlorination equipment from inside may be provided by the installation of glass window(s) in the walls of the chlorine room. Windows should be at least 0.20 m2 in area, and be made of clear wire reinforced glass. There should also be a ***'panic bar'*** on the inside of the chlorine room door for emergency exit.

Storage of Chlorine Cylinders
If necessary, a separate storage room may be provided to simply store the chlorine gas cylinders, with no connection to the line. The chlorine cylinder storage room shall have access either to the chlorine room or from the plant exterior, and arranged to prevent the uncontrolled release of spilled gas.

The chlorine gas storage room shall have provision for ventilation at thirty air changes per hour. Viewing glass windows and panic button on the inside of door should also be provided. In very large facilities, entry into the chlorine rooms may be through a vestibule from outside.

Scrubbers
For facilities located within residential or densely populated areas, consideration shall be given to provide scrubbers for the chlorine room.

Some WWT plants transfer and store chlorine from tankers to holding tanks as shown in the above photograph.

Chlorine Demand

Chlorine combines with a wide variety of materials. These side reactions complicate the use of chlorine for disinfecting purposes. Their demand for chlorine must be satisfied before chlorine becomes available to accomplish disinfection. Amount of chlorine required to react on various water impurities before a residual is obtained. Also, means the amount of chlorine required to produce a free chlorine residual of 0.1 mg/l after a contact time of fifteen minutes as measured by iodmetic method of a sample at a temperature of twenty degrees in conformance with Standard methods.

Chlorine Questions and Answer Review

True or False. Even brief exposure to 1,000 ppm of Cl_2 can be fatal. True

How does one determine the ambient temperature in a chlorine room? Use a regular thermometer because ambient temperature is simply the air temperature of the room.

How is the effectiveness of disinfection determined? From the results of coliform testing.

How often should chlorine storage ventilation equipment be checked? Daily.

Alternate Disinfectants

Chloramine
Chloramine is a very weak disinfectant for Giardia and virus reduction; it is recommended that it be used in conjunction with a stronger disinfectant. It is best utilized as a stable distribution system disinfectant.

In the production of chloramines, the ammonia residuals in the finished water, when fed in excess of stoichiometric amount needed, should be limited to inhibit growth of nitrifying bacteria.

Chlorine Dioxide
Chlorine dioxide may be used for taste and odor control, or as a pre-disinfectant. Total residual oxidants (including chlorine dioxide and chlorite, but excluding chlorate) shall not exceed 0.30 mg/L during normal operation or 0.50 mg/L (including chlorine dioxide, chlorite and chlorate) during periods of extreme variations in the raw water supply.

Chlorine dioxide provides good Giardia and virus protection but its use is limited by the restriction on the maximum residual of 0.5 mg/L ClO_2/chlorite/chlorate allowed in finished water. This limits usable residuals of chlorine dioxide at the end of a process unit to less than 0.5 mg/L.

Where chlorine dioxide is approved for use as an oxidant, the preferred method of generation is to entrain chlorine gas into a packed reaction chamber with a 25% aqueous solution of sodium chlorite ($NaClO_2$).

Warning: Dry sodium chlorite is explosive and can cause fires in feed equipment if leaking solutions or spills are allowed to dry out.

Ozone
Ozone is a very effective disinfectant for both Giardia and viruses. Ozone CT (Contact Time) values must be determined for the ozone basin alone; an accurate T10 value must be obtained for the contact chamber, residual levels measured through the chamber and an average ozone residual calculated.

Ozone does not provide a system residual and should be used as a primary disinfectant only in conjunction with free and/or combined chlorine.

Ozone does not produce chlorinated byproducts (such as trihalomethanes) but it may cause an increase in such byproduct formation if it is fed ahead of free chlorine; ozone may also produce its own oxygenated byproducts such as aldehydes, ketones, or carboxylic acids. Any installed ozonation system must include adequate ozone leak detection alarm systems, and an ozone off-gas destruction system.

Ozone may also be used as an oxidant for removal of taste and odor, or may be applied as a pre-disinfectant.

Ozone

Ozone (O_3) is probably the strongest oxidizing agent available for water treatment. Although it is widely used throughout the world, it has not found much application in the United States. Ozone is obtained by passing a flow of air or oxygen between two electrodes that are subjected to an alternating current in the order of 10,000 to 20,000 volts.

$$3O_2 + \text{electrical discharge} \rightarrow 2O_3$$

Liquid ozone is very unstable and can readily explode. As a result, it is not shipped and must be manufactured on-site. Ozone is a light blue gas at room temperature.
It has a self-policing pungent odor similar to that sometimes noticed during and after heavy electrical storms. In use, ozone breaks down into oxygen and nascent oxygen.

$$O_3 \rightarrow O_2 + O$$

It is the nascent oxygen that produces the high oxidation and disinfections, and even sterilization. Each water has its own ozone demand, in the order of 0.5 ppm to 5.0 ppm. Contact time, temperature, and pH of the water are factors to be determined.

Ozone acts as a complete disinfectant. It is an excellent aid to the flocculation and coagulation process, and will remove practically all color, taste, odor, iron, and manganese. It does not form chloramines or THMs, and while it may destroy some THMs, it may produce others when followed by chlorination. Ozone is not practical for complete removal of chlorine or chloramines, or of THM and other inorganics. Further, because of the possibility of formation of other carcinogens (such as aldehydes or phthalates) it falls into the same category as other disinfectants in that it can produce DBPs.

Ozone generator

Ultraviolet Radiation

The enormous temperatures on the sun create ultraviolet (**UV**) rays in great amounts, and this radiation is so powerful that all life on earth would be destroyed if these ray were not scattered by the atmosphere and filtered out by the layers of ozone gas that float some 20 miles above the earth.

This radiation can be artificially produced by sending strong electric currents through various substances. A sun lamp, for example, sends out UV rays that, when properly controlled, result in a suntan. Of course, too much UV will cause sunburn.

Open Channel UV Lamp
The UV lamp that can be used for the disinfection of water depends upon the low-pressure mercury vapor lamp to produce the ultraviolet energy. A mercury vapor lamp is one in which an electric arc is passed through an inert gas. This in turn will vaporize the mercury contained in the lamp; and it is a result of this vaporization that UV rays are produced.

Enclosed UV lamp assembly. Assemblies will often need frequent cleaning and bulb replacements, there are facilities with 1,000's of bulbs.

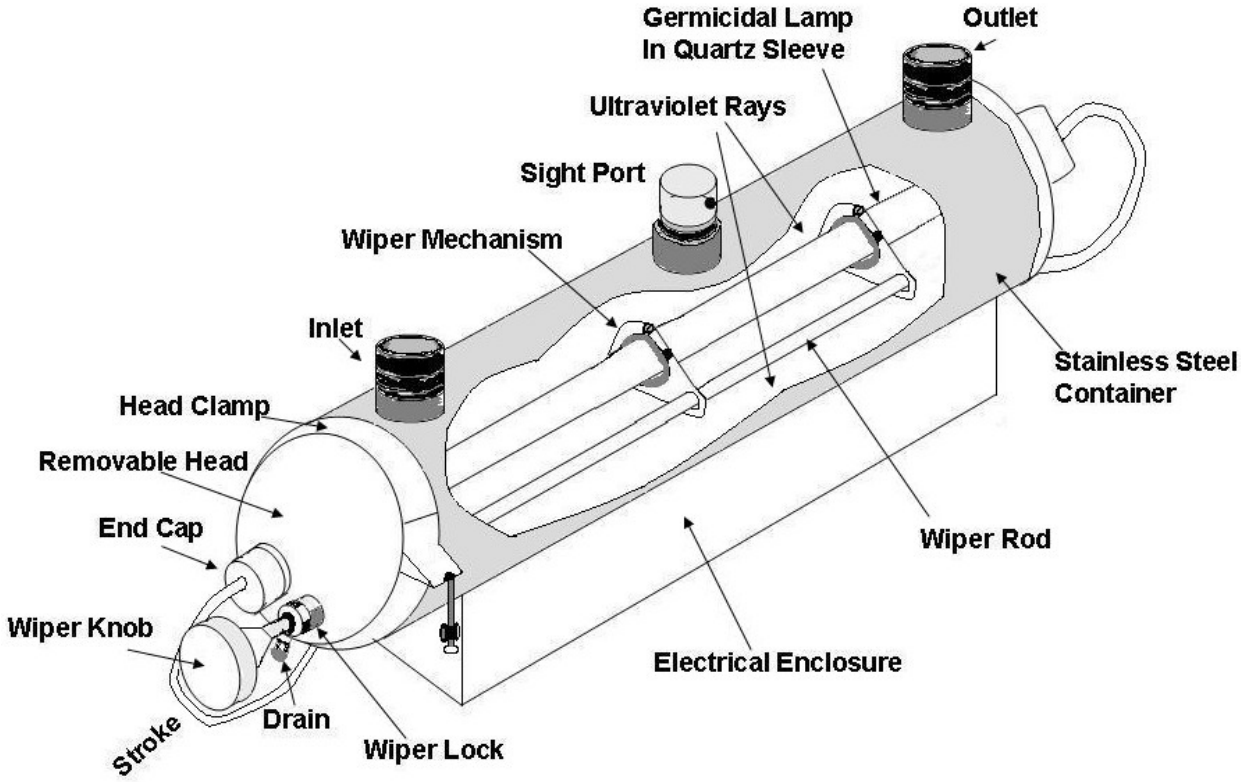
The lamp itself does not come into with contact water, the lamp is placed inside a quartz tube, and the water is in contact with the outside of the quartz tube. Quartz is used in this case since practically none of the UV rays are absorbed by the quartz, allowing all of the rays to reach the water. Ordinary glass cannot be used since it will absorb the UV rays, leaving little for disinfection.

The water flows around the quartz tube. The UV sterilizer will consist of a various number of lamps and tubes, depending upon the quantity of water to be treated. As water enters the sterilizer, it is given a tangential flow pattern so that the water spins over and around the quartz sleeves. In this way the microorganisms spend maximum time and contact with the outside of the quartz tube and the source of the UV rays.

The basic design flow of water of certain UV units is in the order of 2.0 gpm for each inch of the lamp. Further, the units are designed so that the contact or retention time of the water in the unit is not less than 15 seconds. Most manufacturers claim that the UV lamps have a life of about 7,500 hours, which is about 1 year's time. The lamp must be replaced when it loses about 40% to 50% of its UV output; in any installation this is determined by means of a photoelectric cell and a meter that shows the output of the lamp. Each lamp is outfitted with its own photoelectric cell, and with its own alarm that will be activated when the penetration drops to a present level.

Ultraviolet radiation is an excellent disinfectant that is highly effective against viruses, molds, and yeasts; and it is safe to use. It adds no chemicals to the water, it leaves no residual, and it does not form THMs. It is used to remove traces of ozone and chloramines from the finished water. Alone, UV radiation will not remove precursors, but in combination with ozone, it is said to be effective in the removal of THM precursors and THMs.

The germicidal effect of UV is thought to be associated with its absorption by various organic components essential to the cell's functioning. For effective use of ultraviolet, the water to be disinfected must be clean, and free of any suspended solids. The water must also be colorless and must be free of any colloids, iron, manganese, taste, and odor.



These are conditions that must be met. Also, although a water may appear to be clear, such substances as excesses of chlorides, bicarbonates, and sulfates affect absorption of the ultraviolet ray.

These parameters will probably require at least filtration of one type or another. The UV manufacturer will of course stipulate which pretreatment may be necessary.

Removal of Disinfection By-products

Disinfectant	***Disinfectant By-product***	***Disinfectant By-product Removal***
Chlorine (HOCl)	Trihalomethane (THM) Chloramines Chloroprene	Granular Activated Carbon (GAC), resins, controlled coagulation, aeration. GAC-UV GAC
Chloramines (Ch_icl_y)	Probably no THM Others?	GAC UV?
Chlorine dioxide (ClO_2)	Chlorites Chlorates	Use of Fe2+ in coagulation, RO, ion-exchange
Permanganate ($KMnO_4$)	No THMs	
Ozone (O_3)	Aldehydes, Carboxylics, Phthalates	GAC
Ultraviolet (UV)	None known	GAC

The table indicates that most of the disinfectants will leave a by-product that is or would possibly be inimical to health. This may aid with a decision as to whether or not precursors should be removed before these disinfectants are added to water.

If it is decided that removal of precursors is needed, research to date indicates that this removal can be attained through the application of controlled chlorination plus coagulation and filtration, aeration, reverse osmosis, nanofiltration, GAC (Granular Activated Charcoal) or combinations of others processes.

Chlorine Exposure Limits and Final Chlorine Review

This information is necessary to pass your certification exam.

*** OSHA PEL 1 PPM - IDLH 10 PPM and Fatal Exposure Limit 1,000 PPM**

The current Occupational Safety and Health Administration (**OSHA**) permissible exposure limit (**PEL**) for chlorine is 1 ppm (3 milligrams per cubic meter ($mg/m^{(3)}$)) as a ceiling limit. A worker's exposure to chlorine shall at no time exceed this ceiling level. *** IDLH 10 PPM**

Physical and chemical properties of chlorine; a yellowish green, nonflammable and liquefied gas with an unpleasant and irritating smell. It can be readily compressed into a clear, amber-colored liquid, a noncombustible gas, and a strong oxidizer. Solid Chlorine is about 1.5 times heavier than water and gaseous chlorine is about 2.5 times heavier than air. Atomic number of Chlorine is 17. Cl is the elemental symbol and Cl_2 is the chemical formula.

Pairs of substances that chlorine will react explosively or form explosive compounds are Acetylene and ether, turpentine and ammonia and hydrogen and finely divided metals.

Monochloramine, dichloramine, and trichloramine are also known as Combined Available Chlorine. $Cl_2 + NH_4$.

HOCl and OCl^-: The **OCL^-** is the hypochlorite ion and both of these species are known as free available chlorine; they are the two main chemical species formed by chlorine in water. These are known collectively as hypochlorous acid and the hypochlorite ion. When chlorine gas is added to water, it rapidly hydrolyzes. The chemical equations best describes this reaction is **$Cl_2 + H_2O$ --> H+ + Cl- + HOCl.** Hypochlorous acid is the most germicidal of the chlorine compounds with the possible exception of chlorine dioxide.

Yoke-type connectors should be used on a chlorine cylinder's valve, assuming that the threads on the valve may be worn.

The connection from a chlorine cylinder to a chlorinator should be replaced by using a new, approved gasket on the connector. Always follow your manufacturer's instructions.

On a 1 ton Chlorine gas container, the chlorine pressure reducing valve should be located downstream of the evaporator when using an evaporator. This is the liquid chlorine supply line and it is going to be made into Chlorine gas.

In wastewater treatment, Chlorine is added to the effluent before the contact chamber (before the clear well) for complete mixing. One reason for not adding it directly to the chamber is that the chamber has very little mixing due to low velocities.

Here are several safety precautions when using chlorine gas. In addition to protective clothing and goggles, chlorine gas should be used only in a well-ventilated area so that any leaking gas cannot concentrate. Emergency procedures in the case of a large uncontrolled chlorine leak: notify local emergency response team, warn and evacuate people in adjacent areas, and be sure that no one enters the leak area without adequate self-contained breathing equipment.

Here are several symptoms of chlorine exposure: burning of eyes, nose, and mouth, coughing, sneezing, choking, nausea and vomiting; headaches and dizziness; fatal pulmonary edema, pneumonia, and skin blisters. A little Cl_2 will corrode the teeth and then progress to throat cancer.

Approved method for storing a 150 - 200 pound chlorine cylinder: Secure each cylinder in an upright position, attach the protective bonnet over the valve, and firmly secure each cylinder. Never store near heat. Always store the empty in an upright, secure position with proper signage.

Respiratory Protection Section

Conditions for Respirator Use
Good industrial hygiene practice requires that engineering controls be used where feasible to reduce workplace concentrations of hazardous materials to the prescribed exposure limit.

However, some situations may require the use of respirators to control exposure. Respirators must be worn if the ambient concentration of chlorine exceeds prescribed exposure limits. Respirators may be used before engineering controls have been installed, during work operations such as maintenance or repair activities that involve unknown exposures, during operations that require entry into tanks or closed vessels, and during emergencies. Workers should only use respirators that have been approved by NIOSH and the Mine Safety and Health Administration (MSHA).

Respiratory Protection Program
Employers should institute a complete respiratory protection program that, at a minimum, complies with the requirements of OSHA's Respiratory Protection Standard [29 CFR 1910.134]. Such a program must include respirator selection, an evaluation of the worker's ability to perform the work while wearing a respirator, the regular training of personnel; respirator fit testing, periodic workplace monitoring, and regular respirator maintenance, inspection, and cleaning. The implementation of an adequate respiratory protection program (including selection of the correct respirator) requires that a knowledgeable person be in charge of the program and that the program be evaluated regularly.

For additional information on the selection and use of respirators and on the medical screening of respirator users, consult the latest edition of the NIOSH Respirator Decision Logic [NIOSH 1987b] and the NIOSH Guide to Industrial Respiratory Protection [NIOSH 1987a].

Personal Protective Equipment
Workers should use appropriate personal protective clothing and equipment that must be carefully selected, used, and maintained to be effective in preventing skin contact with chlorine. The selection of the appropriate personal protective equipment (**PPE**) (i.e., gloves, sleeves, encapsulating suits) should be based on the extent of the worker's potential exposure to chlorine.

The resistance of various materials to permeation by chlorine liquid and chlorine gas is shown below:

Material Breakthrough Time (hr.) Chlorine Liquid Responder
To evaluate the use of PPE materials with chlorine, users should consult the best available performance data and manufacturers' recommendations. Significant differences have been demonstrated in the chemical resistance of generically similar PPE materials (e.g., butyl) produced by different manufacturers. In addition, the chemical resistance of a mixture may be significantly different from that of any of its neat components.

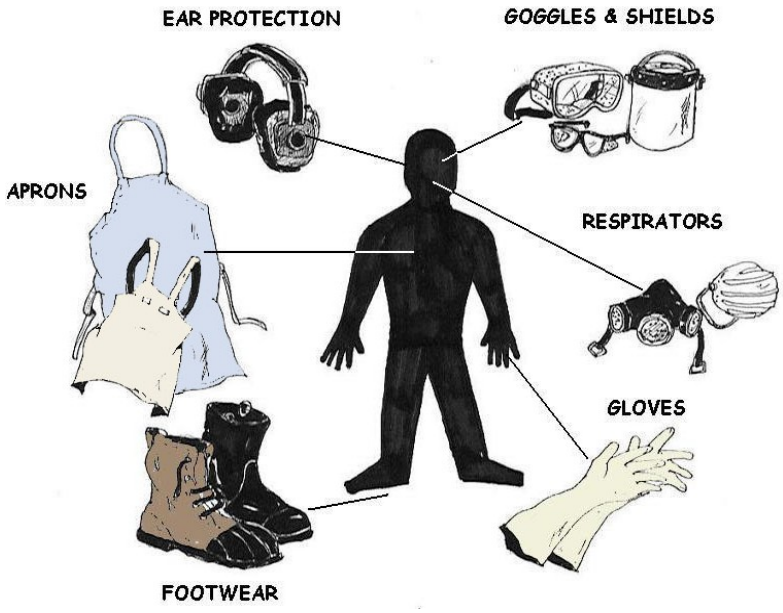
Any chemical-resistant clothing that is used should be periodically evaluated to determine its effectiveness in preventing dermal contact. Safety showers and eye wash stations should be located close to operations that involve chlorine.

Splash-proof chemical safety goggles or face shields (20 to 30 cm long, minimum) should be worn during any operation in which a solvent, caustic, or other toxic substance may be splashed into the eyes. In addition to the possible need for wearing protective outer apparel e.g., aprons, encapsulating suits), workers should wear work uniforms, coveralls, or similar full-body coverings that are laundered each day. Employers should provide lockers or other closed areas to store work and street clothing separately.

Employers should collect work clothing at the end of each work shift and provide for its laundering. Laundry personnel should be informed about the potential hazards of handling contaminated clothing and instructed about measures to minimize their health risk.

Protective clothing should be kept free of oil and grease and should be inspected and maintained regularly to preserve its effectiveness. Protective clothing may interfere with the body's heat dissipation, especially during hot weather or during work in hot or poorly ventilated work environments.

SCBA Fit Test, insuring an air tight fit.



Disinfection Summary

Chlorine

Upon adding chlorine to water, two chemical species, known together as free chlorine, are formed. These species, hypochlorous acid (HOCl, electrically neutral) and hypochlorite ion (OCl-, electrically negative), behave very differently. Hypochlorous acid is not only more reactive than the hypochlorite ion, but is also a stronger disinfectant and oxidant.

The ratio of hypochlorous acid to hypochlorite ion in water is determined by the pH. At low pH (higher acidity), hypochlorous acid dominates while at high pH hypochlorite ion dominates. Thus, the speed and efficacy of chlorine disinfection against pathogens may be affected by the pH of the water being treated. Fortunately, bacteria and viruses are relatively easy targets of chlorination over a wide range of pH. However, treatment operators of surface water systems treating raw water contaminated by the parasitic protozoan Giardia may take advantage of the pH-hypochlorous acid relationship and adjust the pH to be effective against Giardia, which is much more resistant to chlorination than either viruses or bacteria.

Another reason for maintaining a predominance of hypochlorous acid during treatment has to do with the fact that pathogen surfaces carry a natural negative electrical charge. These surfaces are more readily penetrated by the uncharged, electrically neutral hypochlorous acid than the negatively charged hypochlorite ion. Moving through slime coatings, cell walls and resistant shells of waterborne microorganisms, hypochlorous acid effectively destroys these pathogens. Water is made microbiologically safe as pathogens either die or are rendered incapable of reproducing. A typical bacterium has a negatively charged slime coating on its exterior cell wall, which is effectively penetrated by electrically neutral hypochlorous acid, favored by lower pH's.

Factors in Chlorine Disinfection: Concentration and Contact Time

In an attempt to establish more structured operating criteria for water treatment disinfection, the CXT concept came into use in 1980. Based on the work of several researchers, CXT values [final free chlorine concentration (mg/L) multiplied by minimum contact time (minutes)], offer water operators guidance in computing an effective combination of chlorine concentration and chlorine contact time required to achieve disinfection of water at a given temperature. The CXT formula demonstrates that if an operator chooses to decrease the chlorine concentration, the required contact time must be lengthened. Similarly, as higher strength chlorine solutions are used, contact times may be reduced (Connell, 1996).

Chloramines

Chloramines are chemical compounds formed by combining a specific ratio of chlorine and ammonia in water. Because chloramines are relatively weak as a disinfectant, they are almost never used as a primary disinfectant. Chloramines provide a durable residual, and are often used as a secondary disinfectant for long distribution lines and where free chlorine demand is high. Chloramines may also be used instead of chlorine in order to reduce chlorinated byproduct formation and to remove some taste and odor problems.

Advantages

- Reduced formation of THMs, HAAs
- Will not oxidize bromide to bromine forming brominated byproducts
- More stable residual than free chlorine
- Excellent secondary disinfectant, has been found to be better than free chlorine at controlling coliform bacteria and biofilm growth
- Lower taste and odor than free chlorine

Limitations

- Weak disinfectant and oxidant
- Requires shipment and handling of ammonia or ammonia compounds as well as chlorinating chemicals
- Ammonia is toxic to fish, and may pose problems for aquarium owners
- Will cause problems for kidney dialysis if not removed from water

Chlorine Dioxide

Chlorine dioxide (ClO_2) is generated on-site at water treatment facilities. In most generators sodium chlorite and elemental chlorine are mixed in solution, which almost instantaneously forms chlorine dioxide. Chlorine dioxide characteristics are quite different from chlorine. In solution it is a dissolved gas, which makes it largely unaffected by pH but volatile and relatively easily stripped from solution. Chlorine dioxide is also a strong disinfectant and a selective oxidant. While chlorine dioxide does produce a residual it is only rarely used for this purpose.

Advantages

- ✓ Effective against Cryptosporidium
- ✓ Up to five times faster than chlorine at inactivating Giardia
- ✓ Disinfection is only moderately affected by pH
- ✓ Will not form chlorinated byproducts (THMs, HAAs)
- ✓ Does not oxidize bromide to bromine (can form bromate in sunlight)
- ✓ More effective than chlorine in treating some taste and odor problems
- ✓ Selective oxidant used for manganese oxidation and targeting some chlorine resistant organics

Limitations

- ✓ Inorganic byproduct formation (chlorite, chlorate)
- ✓ Highly volatile residuals
- ✓ Requires on-site generation equipment and handling of chemicals (chlorine and sodium chlorite)
- ✓ Requires a high level of technical competence to operate and monitoring equipment, product and residuals
- ✓ Occasionally poses unique odor and taste problems
- ✓ High operating cost (chlorite chemical cost is high)

Understanding Chlorine Basics

Chlorine is applied to water in one of three forms: elemental chlorine (chlorine gas), hypochlorite solution (bleach), or dry calcium hypochlorite. All three forms produce free chlorine in water.

Advantages

- ✓ Highly effective against most pathogens
- ✓ Provides a residual to protect against recontamination and to reduce bio-film growth in the distribution system
- ✓ Easily applied, controlled, and monitored
- ✓ Strong oxidant meeting most preoxidation objectives
- ✓ Operationally the most reliable
- ✓ The most cost-effective disinfectant

Limitations

- ✓ Byproduct formation (THMs, HAAs)
- ✓ Will oxidize bromide to bromine, forming brominated organic byproducts
- ✓ Not effective against Cryptosporidium
- ✓ Requires transport and storage of chemicals

Elemental Chlorine

Elemental chlorine is the most commonly used form of chlorine. It is transported and stored as a liquefied gas under pressure. Water treatment facilities typically use chlorine in 100 and 150-lb cylinders or one-ton containers. Some large systems use railroad tank cars or tanker trucks.

Advantages

- ✓ Lowest cost of chlorine forms
- ✓ Unlimited shelf-life

Limitations

- ✓ Hazardous gas requires special handling and operator training
- ✓ Additional regulatory requirements, including EPA's Risk Management Program and the Occupational Safety and Health Administration's Process Safety Management program

Factors in Chlorine Disinfection: Concentration and Contact Time

In an attempt to establish more structured operating criteria for water treatment disinfection, the CXT concept came into use in 1980. Based on the work of several researchers, CXT values [final free chlorine concentration (mg/L) multiplied by minimum contact time (minutes)], offer water operators guidance in computing an effective combination of chlorine concentration and chlorine contact time required to achieve disinfection of water at a given temperature. The CXT formula demonstrates that if an operator chooses to decrease the chlorine concentration, the required contact time must be lengthened. Similarly, as higher strength chlorine solutions are used, contact times may be reduced (Connell, 1996).

Disinfection and Bioterrorism
Disinfection is crucial to water system security, providing the 'front line' of defense against biological contamination. Normal filtration and disinfection processes would dampen or remove the threats posed by a number of potential bioterrorism agents. In addition, water systems should maintain an ability to increase disinfection doses in response to a particular threat.
However, conventional treatment barriers in no way guarantee safety from biological attacks. For many potential bioterrorism agents, there is little scientific information about what levels of reduction can be achieved with chlorine or other disinfectants. In addition, contamination of water after it is treated could overwhelm the residual disinfectant levels in distribution systems. Furthermore, typical water quality monitoring does not provide real-time data to warn of potential problems (Rose 2002). Additional research and funding are needed to improve prevention, detection, and responses to potential threats.

Protecting Chlorine and Other Treatment Chemicals
As part of its vulnerability assessment, each water system must consider its transportation, storage and use of treatment chemicals. These chemicals are both critical assets (necessary for delivering safe water) and potential vulnerabilities (may pose significant hazards, if released). For example, a release of chlorine gas would pose an immediate threat to system operators, and a large release may pose a danger to the surrounding community. As part of its vulnerability assessment, a water system using chlorine must determine if existing layers of protection are adequate. If not, a system should consider additional measures to reduce the likelihood of an attack or to mitigate the potential consequences.

Possible measures to address chlorine security include: enhanced physical barriers (e.g., constructing secure chemical storage facilities), policy changes (e.g., tightening procedures for receiving chemical shipments), reducing quantities stored on site, or adopting alternative disinfection methods. These options must be weighed and prioritized, considering the unique characteristics and resources of each system. Water system officials must evaluate the risk-tradeoffs associated with each option. For example, reducing the chemical quantities on-site may reduce a system's ability to cope with an interruption of chemical supplies. Furthermore, changing disinfection technologies will not necessarily improve overall safety and security.

Understanding Calculation and Reporting of CT Data
Basically, log inactivation is a measurement of how effective a disinfection process is at killing microorganisms in a specific environment. Operationally, directly measuring log inactivation is not practical, but determining the microbial inactivation for an individual water treatment plant (WTP) can be achieved using the log inactivation calculations. The log inactivation calculation adjusts the WTP's CT value to account for the disinfection chemical reaction process variables that influence the disinfection process efficiency.

Log Inactivation
"Log inactivation" is a convenient way to express the number or percent of microorganisms inactivated (killed or unable to replicate) through the disinfection process. For example, a 3 log inactivation value means that 99.9% of microorganisms of interest have been inactivated. Log inactivation measures the effectiveness of the disinfection process, which is influenced by variables including disinfectant concentration, temperature, pH and disinfectant type (e.g., lower temperature results in less inactivation since the reactions slow down as temperature decreases).

CT and Log Inactivation Calculation Overview

This reference takes you step by step through the CT and log inactivation calculation procedure, through an example calculation, and presents the disinfection segment concept.

"CT" (minutes•mg/L) in the context of water treatment is defined as the product of: C, for "residual disinfectant concentration" in mg/L (determined before or at the first customer) and T, for the corresponding "disinfectant contact time" in minutes. CT is a measure of the disinfection process reaction time, but CT is only one of several variables that control the effectiveness of the disinfection
process.

CTCALC = Concentration Time, Calculated Value (minutes•mg/L)

C = Residual disinfectant concentration measured during peak flow (mg/L)

T = Actual Detention Time (minutes)

CTCALC = C × T

TDT = Theoretical Detention Time (minutes)

V = Volume, based on low water level (gallons)

Q = Peak hourly flow (gpm)

TDT = V/Q

Volume Equations:
Cylindrical: π x r2 x d Pipeline: π x r2 x l
Rectangular: l x w x d
d = minimum water depth
π = 3.1416

Disinfection Segments
Total inactivation = Σ log inactivation from each disinfection segment
Disinfection Profile
Almost all community and non-transient, non-community public water systems that use Surface Water or Ground Water Under the Direct Influence of Surface Water sources are required to develop a disinfection profile. Systems are required to retain the disinfection profile in graphic form and it must be available for review by the state as part of a sanitary survey.

Disinfection Profile and Benchmark
• A disinfection profile is a graphical representation of a system's level of *Giardia lamblia* or virus inactivation measured, at least weekly, during the course of a year.
• A benchmark is the lowest monthly average microbial inactivation during the disinfection profile time period.

The EPA has developed a disinfection profile spreadsheet calculator that calculates and graphs the disinfection profile for *Giardia* and viruses. The spreadsheet can be downloaded from: http://www.epa.gov/safewater/mdbp/lt1eswtr.html.

Understanding Chlorine Demand
The amount of chlorine used by reactions with substances that oxidize in the water before chlorine residual can be measured. It is the difference between the amount of chlorine added to wastewater and the amount of chlorine residual remaining after a given contact time. Chlorine demand may change with dosage, time, temperature, pH, and the type and amount of pollutants in the water.

The presence of chlorine residual in drinking water indicates that: 1) a sufficient amount of chlorine was added initially to the water to inactivate the bacteria and some viruses that cause diarrheal disease; and, 2) the water is protected from recontamination during storage. The presence of free residual chlorine in drinking water is correlated with the absence of disease-causing organisms, and thus is a measure of the potability of water.

While chlorine's most important attributes are its broad-spectrum germicidal potency and persistence in water distribution systems, its ability to efficiently and economically address many other water treatment concerns has also supported its wide use. Chlorine-based compounds are the only major disinfectants exhibiting lasting residual properties. Residual protection guards against microbial regrowth and prevents contamination of the water as it moves from the treatment plant to household taps.

Definitions
When chlorine is added to water, some of the chlorine reacts first with organic materials and metals in the water and is not available for disinfection (this is called the chlorine demand of the water). The remaining chlorine concentration after the chlorine demand is accounted for is called total chlorine. Total chlorine is further divided into: 1) the amount of chlorine that has reacted with nitrates and is unavailable for disinfection which is called combined chlorine and, 2) the free chlorine, which is the chlorine available to inactivate disease-causing organisms, and thus a measure to determine the potability of water.

For example, if using completing clean water the chlorine demand will be zero, and there will be no nitrates present, so no combined chlorine will be present. Thus, the free chlorine concentration will be equal to the concentration of chlorine initially added. In natural waters, especially surface water supplies such as rivers, organic material will exert a chlorine demand, and nitrates will form combined chlorine. Thus, the free chlorine concentration will be less than the concentration of chlorine initially added.

Chlorine Dose, Demand, and Residual
Most water treatment plants are required to disinfect the water, a process used to kill harmful bacteria. The most frequently used method of disinfection is the addition of chlorine. Here, we will briefly introduce three terms used during chlorination - chlorine dose, chlorine demand, and chlorine residual. These three characteristics are related to each other using the following equation:

(Chlorine demand) = (Chlorine dose) - (Chlorine residual)

The amount of chlorine added to the water is known as the chlorine dose. This is a measured quantity chosen by the operator and introduced into the water using a chlorinator or hypochlorinator.

As the chlorine reacts with bacteria and chemicals in the water, some of the chlorine is used up. The amount of chlorine used up by reacting with substances in the water is known as the chlorine demand. If nothing reacts with the chlorine (as would be the case in distilled water), then the chlorine demand is zero. However, in most cases the operator should count on some of the chlorine dose being used up when it reacts with substances in the water.

The amount of chlorine remaining in the water after some of the chlorine reacts with substances in the water is known as the chlorine residual. This lab introduces a test which can be used to calculate the chlorine residual. The chlorine residual is the most important of these three values - dose, demand, and residual - because it represents the actual amount of chlorine remaining in the water to act as a disinfectant.

The test for chlorine residual is performed frequently at most water treatment plants. Since regulations require a certain level of chlorine in water at the far ends of the distribution system, operators should be sure to test the chlorine residual in the distribution system as well as in the clear well.

Combined residual chlorination involves the addition of chlorine to water to produce, with natural ammonia present or with ammonia added, a combined available chlorine residual. Combined available chlorine forms have lower oxidation potentials than free available chlorine forms and are less effective as oxidants. They are also less effective as disinfectants. In fact, 25 times more combined available residual chlorine must be obtained to meet the same disinfectant level as a free available residual. The contact time has to be up to 100 times greater to obtain the same level of bacterial kill at the same pH and temperature conditions.

When a combined available chlorine residual is desired, the character of the water determines how it can be accomplished. These conditions may have to be considered:
1. If the water contains sufficient ammonia to produce the desired level of combined residual, the application of sufficient chlorine alone is all that is needed.
2. If the water contains too little or no ammonia, then addition of both chlorine and ammonia is required.
3. If the water has a free available chlorine, the addition of ammonia alone is all that is required. A combined chlorine residual should contain little or no free available chlorine.

The practice of combined residual chlorination is the most effective way of maintaining a stable residual throughout the distribution system to the point of consumer use. Combined residuals in the distribution system are generally longer-lasting and will carry farther into the system, but they are not as effective as free residuals are at disinfecting. The levels required by the regulatory agencies, when using combined residuals, is 1.0 ppm to 2.0 ppm.

Understanding Chlorine Residual
The amount of available chlorine present in wastewater after a given contact time (20 minutes at peak flow; 30 minutes at average flow), and under specific conditions including pH and temperature.

For effective water treatment, the water supply industry has recognized the need for adequate exposure to the disinfectant and sufficient disinfectant dosage for a certain amount of time. In the 1980s, the two functions were combined with the development of the CT values for various disinfectants.

CT represents the combination of the disinfectant dosage and the length of time water has been exposed to a minimum amount of the disinfectant residual.

Mathematically it is represented as CT = concentration x time
concentration = final disinfectant concentration in mg/l
time = minimum exposure time in minutes

In an assessment of disinfection effectiveness, two types of organisms have been chosen as disinfection surrogates – the protozoan Giardia and viruses. CT values established for disinfection of surface waters require treatment plants to achieve a three-log or 99.9% reduction in Giardia and a four-log or 99.99% virus reduction. It is important to recognize that the use of chlorine as the disinfectant is only one part of the treatment process. Equally important is the

need for improved filtration to remove organisms. A combination of proper disinfection and filtration is most effective in providing safe drinking water. Recent experiments in controlling Cryptosporidium also suggest the effectiveness of filtration in the water treatment process.

Free residual chlorination involves the application of chlorine to water to produce--either directly or by first destroying any naturally present ammonia--a free available chlorine residual and to maintain this residual through part or all of the water treatment plant and distribution system. Free available residual forms have higher oxidation potentials than combined available chlorine forms and are more effective as disinfectants.

When free available chlorine residuals are desired, the characteristics of the water will determine how this will be accomplished. This may have to be considered:
1. If the water contains no ammonia or other nitrogen compounds, any application of chlorine will yield a free residual once it has reacted with any bacteria, virus and other microorganisms present in the water.
2. If the water contains ammonia, it results in the formation of a combined residual, which must be destroyed by applying an excess of chlorine.

Breakpoint Chlorination
Breakpoint chlorination is the name of the process of adding chlorine to water until the chlorine demand has been satisfied. Chlorine demand equals the amount of chlorine used up before a free available chlorine residual is produced. Further additions of chlorine will result in a chlorine residual that is directly proportional to the amount of chlorine added beyond the breakpoint. Public water supplies normally chlorinate past the breakpoint.

When chlorine is initially added to water, the following may happen:
1. If the water contains some iron, manganese, organic matter, and ammonia, the chlorine reacts with these materials and no residual is formed, meaning that no disinfection has taken place.
2. If additional chlorine is added at this point, it will react with the organics and ammonia to form chloramines. The chloramines produce a combined chlorine residual. As the chlorine is combined with other substances, it loses some of the disinfection strength. Combined residuals have poor disinfection power and may be the cause of taste and odor problems.
3. With a little more chlorine added, the chloramines and some of the chlororganics are destroyed.
4. With still more chlorine added, a free chlorine residual is formed, free in the sense that it can react quickly.

Free available chlorine is the best residual for disinfection. It disinfects faster and without the swimming-pool odor of combined residual chlorine. The free available residual forms at the breakpoint; therefore, the process is called breakpoint chlorination. The common practice today is to go just beyond the breakpoint to a residual of about .2 to .5 ppm.

A variety of reactions take place during chlorination. When chlorine is added to a water containing ammonia (NH_3), the ammonia reacts with hypochlorous acid (HOCL) to form monochloramine, dichloramine, and trichloramine. The formation of these chloramines depends on the pH of the water and the initial chlorine-ammonia ratio.

Ammonia + Hypochlorous acid ----> Chloramine + Water
NH3 + HOC1----------> NH2C1 + H20 Monochloramine
NH2C1 + HOC1---------- > NHC12 + H20 Dichloramine
NHC12 + HOC1---------- > NC13 + H20 Trichloramine

At the pH of most natural water (pH 6.5 to 7.5), monochloramine and dichloramine exist together. At pH levels below 5.5, dichloramine exists by itself. Below pH 4.0, trichloramine is the only compound found. The monochloramine and dichloramine forms have a definite disinfection power. Dichloramine is a more effective disinfecting agent than monochloramine.

However, dichloramine is not recommended as a disinfectant due to the possibility of the formation of taste and odor compounds. Chlorine reacts with phenol and salicylic acid to form chlorophenol, which has an intense medicinal odor. This reaction is much slower in the presence of monochloramines.

Both the chlorine residual and the contact time are essential for effective disinfection. It is important to have complete mixing. The operator also needs to be aware that changes in the pH may affect the ability of the chlorine to disinfect the water. The operator must examine the application and select the best point of feed and the best contact time to achieve the results desired. The operator needs to consider:
1. Whether the injection point and the method of mixing is designed so that the disinfectant is able to get into contact with all of the water to be disinfected. This also depends on whether pre- and/or post-chlorination is being used.
2. Contact time. In situations of good initial mixing, the longer the contact time, the more effective the disinfection.
3. Effectiveness of upstream treatment processes. The lower the turbidity of the water, the more effective the disinfection.
4. Temperature. At higher temperatures the rate of disinfection is more rapid.
5. Dosage and type of chemical. Usually the higher the dose, the quicker the disinfection rate. The form of disinfectant (chloramine or free chlorine) and the type of chemical used influence the disinfection rate.
6. pH. The lower the pH, the better the disinfection.

Emergency Disinfection of Drinking Water
USE ONLY WATER THAT HAS BEEN PROPERLY DISINFECTED FOR DRINKING, COOKING, MAKING ANY PREPARED DRINK, OR FOR BRUSHING TEETH
1. Use bottled water that has not been exposed to flood waters if it is available.
2. If you don't have bottled water, you should boil water to make it safe. Boiling water will kill most types of disease-causing organisms that may be present. If the water is cloudy, filter it through clean cloths or allow it to settle, and draw off the clear water for boiling. Boil the water for one minute, let it cool, and store it in clean containers with covers.
3. If you can't boil water, you can disinfect it using household bleach. Bleach will kill some, but not all, types of disease-causing organisms that may be in the water. If the water is cloudy, filter it through clean cloths or allow it to settle, and draw off the clear water for disinfection. Add 1/8 teaspoon (or 8 drops) of regular, unscented, liquid household bleach for each gallon of water, stir it well and let it stand for 30 minutes before you use it. Store disinfected water in clean containers with covers.
4. If you have a well that has been flooded, the water should be tested and disinfected after flood waters recede. If you suspect that your well may be contaminated, contact your local or state health department or agriculture extension agent for specific advice.
(U.S. federal agencies and the Red Cross recommend these same four steps to disinfect drinking water in an emergency. Please, read the text below for important details about disinfection.
More information about disinfection

- ✓ In times of crisis, follow advice from local officials. Local health departments or public water systems may urge consumers to use more caution or to follow additional measures than the information provided here.

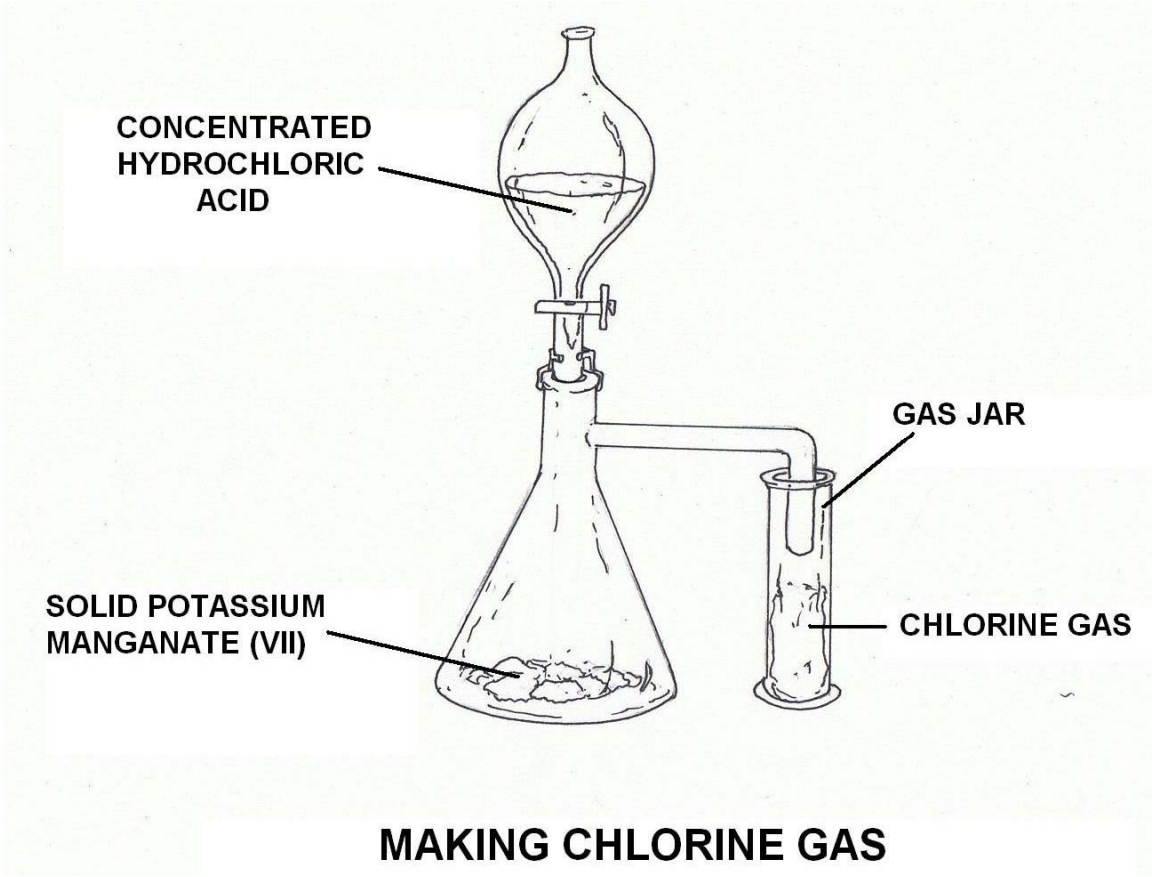
- ✓ Look for other sources of potable water in and around your home.

- ✓ When your home water supply is interrupted by natural or other forms of disaster, you can obtain limited amounts of water by draining your hot water tank or melting ice cubes. In most cases, well water is the preferred source of drinking water. If it is not available and river or lake water must be used, avoid sources containing floating material and water with a dark color or an odor. Generally, flowing water is better quality than stagnant water.

Examine the physical condition of the water.
When emergency disinfection is necessary, disinfectants are less effective in cloudy, murky or colored water. Filter murky or colored water through clean cloths or allow it to settle. It is better to both settle and filter. After filtering until it is clear, or allowing all dirt and other particles to settle, draw off the clean and clear water for disinfection. Water prepared for disinfection should be stored only in clean, tightly covered, containers, not subject to corrosion.

- ✓ Choose a disinfection method.

- ✓ Boiling and chemical treatment are two general methods used to effectively disinfect small quantities of filtered and settled water.



MAKING CHLORINE GAS

Boiling

Boiling is the surest method to make water safe to drink and kill disease-causing microorganisms like Giardia lamblia and Cryptosporidium, which are frequently found in rivers and lakes. These disease-causing organisms are less likely to occur in well water (as long as it has not been affected by flood waters). If not treated properly and neutralized, Giardia may cause diarrhea, fatigue, and cramps after ingestion. Cryptosporidium is highly resistant to disinfection. It may cause diarrhea, nausea and/or stomach cramps. People with severely weakened immune systems are likely to have more severe and more persistent symptoms than healthy individuals.

Boil filtered and settled water vigorously for one minute (at altitudes above one mile, boil for three minutes). To improve the flat taste of boiled water, aerate it by pouring it back and forth from one container to another and allow it to stand for a few hours, or add a pinch of salt for each quart or liter of water boiled.

If boiling is not possible, chemical disinfection of filtered and settled water collected from a well, spring, river, or other surface water body will still provide some health benefits and is better than no treatment at all.

Chemical Treatment
When boiling is not practical, certain chemicals will kill most harmful or disease-causing organisms. For chemical disinfection to be effective, the water must be filtered and settled first. Chlorine and iodine are the two chemicals commonly used to treat water. They are somewhat effective in protecting against exposure to Giardia, but may not be effective in controlling more resistant organisms like Cryptosporidium. Chlorine is generally more effective than iodine in controlling Giardia, and both disinfectants work much better in warm water. You can use a non-scented, household chlorine bleach that contains a chlorine compound to disinfect water. Do not use non-chlorine bleach to disinfect water. Typically, household chlorine bleaches will be 5.25% available chlorine. Follow the procedure written on the label. When the necessary procedure is not given, find the percentage of available chlorine on the label and use the information in the following table as a guide. (Remember, 1/8 teaspoon and 8 drops are about the same quantity.)

Available Chlorine

Drops per Quart/Gallon of Clear Water Drops per Liter of Clear Water
1% 10 per Quart - 40 per Gallon 10 per Liter
4-6% 2 per Quart - 8 per Gallon (1/8 teaspoon) 2 per Liter
7-10% 1 per Quart - 4 per Gallon 1 per Liter

(If the strength of the bleach is unknown, add ten drops per quart or liter of filtered and settled water. Double the amount of chlorine for cloudy, murky or colored water or water that is extremely cold.)

Mix the treated water thoroughly and allow it to stand, preferably covered, for 30 minutes. The water should have a slight chlorine odor. If not, repeat the dosage and allow the water to stand for an additional 15 minutes. If the treated water has too strong a chlorine taste, allow the water to

stand exposed to the air for a few hours or pour it from one clean container to another several times.

You can use granular calcium hypochlorite to disinfect water.
Add and dissolve one heaping teaspoon of high-test granular calcium hypochlorite (approximately ¼ ounce) for each two gallons of water, or 5 milliliters (approximately 7 grams) per 7.5 liters of water. The mixture will produce a stock chlorine solution of approximately 500 milligrams per liter, since the calcium hypochlorite has available chlorine equal to 70 percent of its weight. To disinfect water, add the chlorine solution in the ratio of one part of chlorine solution to each 100 parts of water to be treated. This is roughly equal to adding 1 pint (16 ounces) of stock chlorine to each 12.5 gallons of water or (approximately ½ liter to 50 liters of water) to be disinfected. To remove any objectionable chlorine odor, aerate the disinfected water by pouring it back and forth from one clean container to another.

You can use chlorine tablets to disinfect filtered and settled water.

Chlorine tablets containing the necessary dosage for drinking water disinfection can be purchased in a commercially prepared form. These tablets are available from drug and sporting goods stores and should be used as stated in the instructions. When instructions are not available, use one tablet for each quart or liter of water to be purified.

You can use tincture of iodine to disinfect filtered and settled water.

Common household iodine from the medicine chest or first aid kit may be used to disinfect water. Add five drops of 2 percent U.S. or your country's approved Pharmacopeia tincture of iodine to each quart or liter of clear water. For cloudy water add ten drops and let the solution stand for at least 30 minutes.

You can use iodine tablets to disinfect filtered and settled water.

Purchase commercially prepared iodine tablets containing the necessary dosage for drinking water disinfection at drug and sporting goods stores. Use as stated in instructions. When instructions are not available, use one tablet for each quart or liter of filtered and settled water to be purified.

ONLY USE WATER THAT HAS BEEN PROPERLY DISINFECTED FOR DRINKING, COOKING, MAKING ANY PREPARED DRINK, OR FOR BRUSHING TEETH.

Summary and Illustration of Key Points

- ✓ Filter murky or colored water through clean cloths or allow it to settle. It is better to both settle and filter.

- ✓ Boiling is the surest method to make water safe to drink and kill disease-causing microorganisms like Giardia lamblia and Cryptosporidium, which are frequently found in rivers and lakes.

- ✓ To improve the flat taste of boiled water, aerate it by pouring it back and forth from one container to another and allow it to stand for a few hours, or add a pinch of salt for each quart or liter of water boiled.

- ✓ When boiling is not practical, certain chemicals will kill most harmful or disease-causing organisms. Chlorine (in the form of unscented bleach) and iodine are the two chemicals commonly used to treat water.

- ✓ You can use a non-scented, household chlorine bleach that contains a chlorine compound to disinfect water. (Remember, 1/8 teaspoon and 8 drops are about the same quantity.)
- ✓ You can use tincture of iodine to disinfect filtered and settled water. Common household iodine from the medicine chest or first aid kit may be used to disinfect water.
- ✓ Tincture of iodine. For cloudy water add ten drops and let the solution stand for at least 30 minutes.

Understanding Combined Chlorine Residual

The residual consisting of chlorine that is combined with ammonia, nitrogen, or nitrogenous compounds (chloramines).

Understanding Free Available Chlorine
The residual consisting of hypochlorite ions (OCl-), hypochlorous acid (HOCl) or a combination of the two. These are the most effective in killing bacteria.

Total Combined Chlorine Residual
The total amount of chlorine present in a sample. This is the sum of the free chlorine residual and the combined available chlorine residual.

Understanding Pre-Chlorination
Chlorination is the application of chlorine to water to accomplish some definite purpose. In this lesson, we will be concerned with the application of chlorine for the purpose of disinfection, but you should be aware that chlorination can also be used for taste and odor control, iron and manganese removal, and to remove some gases such as ammonia and hydrogen sulfide. Chlorination is currently the most frequently used form of disinfection in the water treatment field. However, other disinfection processes have been developed. These alternatives will be discussed at the end of this lesson.

Pre-Chlorination and Post-Chlorination
Like several other water treatment processes, chlorination can be used as a pretreatment process (prechlorination) or as part of the primary treatment of water (postchlorination). Treatment usually involves either postchlorination only or a combination of prechlorination and postchlorination.

Pre-chlorination is the act of adding chlorine to the raw water. The residual chlorine is useful in several stages of the treatment process - aiding in coagulation, controlling algae problems in basins, reducing odor problems, and controlling mudball formation. In addition, the chlorine has a much longer contact time when added at the beginning of the treatment process, so prechlorination increases safety in disinfecting heavily contaminated water.

Post-chlorination is the application of chlorine after water has been treated but before the water reaches the distribution system. At this stage, chlorination is meant to kill pathogens and to provide a chlorine residual in the distribution system. Post-chlorination is nearly always part of the treatment process, either used in combination with prechlorination or used as the sole disinfection process.

Until the middle of the 1970s, water treatment plants typically used both prechlorination and post-chlorination. However, the longer contact time provided by prechlorination allows the chlorine to react with the organics in the water and produce carcinogenic substances known as trihalomethanes. As a result of concerns over trihalomethanes, prechlorination has become much less common in the United States. Currently, prechlorination is only used in plants where trihalomethane formation is not a problem.

Understanding Breakpoint Chlorination
Addition of chlorine to water until the chlorine demand has been satisfied. Since ammonia is present in all domestic wastewaters, the reaction of ammonia with chlorine is a great significance. When chlorine is added to waters containing ammonia, the ammonia reacts with hypochlorous acid (HOCl) to form monochloramine, dichloramine and trichloramine. The formation of these chloramines depends on the pH of the solution and the initial chlorine-ammonia ratio.

Chlor-Alkali Membrane Process
The chloralkali process (also chlor-alkali and chlor alkali) is an industrial process for the electrolysis of sodium chloride solution (brine). Depending on the method, several products besides hydrogen can be produced. If the products are separated, chlorine and sodium hydroxide (caustic soda) are the products; by mixing, sodium hypochlorite or sodium chlorate are produced, depending on the temperature. Higher temperatures are needed for the production of sodium chlorate instead of sodium hypochlorite. Industrial scale production began in 1892. When using calcium chloride or potassium chloride, the products contain calcium or potassium instead of sodium.

The process has a high energy consumption, for example over 4 billion kWh per year in West Germany in 1985, and produces equal (molar) amounts of chlorine and sodium hydroxide, which makes it necessary to find a use for the product for which there is less demand, usually the chlorine. There are three production methods in use. While the mercury cell method produces chlorine-free sodium hydroxide, the use of several tons of mercury leads to serious environmental problems. In a normal production cycle a few hundred pounds of mercury per year are emitted, which accumulate in the environment. Additionally, the chlorine and sodium hydroxide produced via the mercury-cell chloralkali process are themselves contaminated with trace amounts of mercury. The membrane and diaphragm method use no mercury, but the sodium hydroxide contains chlorine, which must be removed.

Understanding Chlorine's Effectiveness
In 1881, German bacteriologist Robert Koch demonstrated under controlled laboratory conditions that pure cultures of bacteria could be destroyed by hypochlorite (bleach). The bulk of chlorine disinfection research, which was conducted from the 1940s to the 1970s with a focus on bacteria, provided observations as to how chlorine kills the microorganism. The observations that (1) bacterial cells dosed with chlorine release nucleic acids, proteins and potassium and (2) membrane functions such as respiration and active transport are affected more by chlorine than are cytoplasmic processes, directed researchers' attention to the surface of the bacterial cell. The hypothesis was that the bacterial cell wall, under environmental stress, could interact with chlorine.

Chlorine exposure appears to cause physical, chemical, and biochemical alterations to the cell wall, thus destroying the cell's protective barrier, terminating vital functions, resulting in death of the microorganism. A possible sequence of events during chlorination would be: (1) disruption of the cell wall barrier by reactions of chlorine with target sites at the cell surface, (2) release of vital cellular constituents from the cell, (3) termination of membrane-associated functions, and (4) termination of cellular functions within the cell. During the course of this sequence of events, the microorganism dies, meaning it is no longer capable of growing or causing disease.

Understanding Chlorine Solubility Effects
Chlorine is only slightly soluble in water; its maximum solubility is approximately one percent at 49° C. At temperatures below this point it combines with water to form chlorine ice, a crystalline substance. When the water supply to a gas chlorinator is below normal room temperature, it may cool the chlorine gas to the point at which chlorine ice is formed and accumulates on the needle valve and gas outlet tube, resulting in erratic feed results. Because the vapor pressure of chlorine increases with rising temperatures, its solubility also decreases. At 212° F. chlorine is insoluble in water.

Chlorine dissolved in water forms a weak corrosive mixture of hydrochloric and hypochlorous acid. The corrosivity of chlorine solutions in water creates problems in handling chlorine spills and chlorine containers. Chlorine reacts with many compounds. Because of its great affinity for hydrogen, it removes hydrogen from some compounds, such as hydrogen sulfide. It also reacts with ammonia or other nitrogen-containing compounds to form various mixtures of chloramines. It reacts with organic materials, sometimes with explosive violence.

Chemicals like chlorine, bromine, and ozone are examples of oxidizers. It is their ability to oxidize or steal electrons from other substances that makes them good water sanitizers. As soon as the oxidizing agent is added to the water, it begins to combine with microorganisms like bacteria, algae, and whatever else the water may contain.

Now the free and available oxidizer is combining with contaminants and its effectiveness is reduced according to how much combining took place. Although the hydrogen ion does not play a direct reduction role on copper surfaces, pH can influence copper corrosion by altering the equilibrium potential of the oxygen reduction half-reaction and by changing the speciation of copper in solution (Reiber, 1989). Copper corrosion increases rapidly as the pH drops below 6; in addition, uniform corrosion rates can be high at low pH values (below about pH 7), causing metal thinning. At higher pH values (above about pH 8), copper corrosion problems are almost always associated with non-uniform or pitting corrosion processes (Edwards et al., 1994a; Ferguson et al., 1996). Edwards et al. (1994b) found that for new copper surfaces exposed to simple solutions that contained bicarbonate, chloride, nitrate, perchlorate or sulphate, increasing the pH from 5.5 to 7.0 roughly halved corrosion rates, but further increases in pH yielded only subtle changes.

The prediction of copper levels in drinking water relies on the solubility and physical properties of the cupric oxide, hydroxide and basic carbonate solids that comprise most scales in copper water systems (Schock et al., 1995). In the cupric hydroxide model of Schock et al. (1995), a decrease in copper solubility with higher pH is evident. Above a pH of approximately 9.5, an upturn in solubility is predicted, caused by carbonate and hydroxide complexes increasing the solubility of cupric hydroxide. Examination of experience from 361 utilities reporting copper levels under the U.S. EPA Lead and Copper Rule revealed that the average 90th-percentile copper levels were highest in waters with pH below 7.4 and that no utilities with pH above 7.8 exceeded the U.S. EPA's action level for copper of 1.3 mg/L (Dodrill and Edwards, 1995). However, problems associated with copper solubility were also found to persist up to about pH 7.9 in cold, high-alkalinity and high-sulphate groundwater (Edwards et al., 1994a).

In the pH range of 7-9, both the corrosion rate and the degree of tuberculation of iron distribution systems generally increase with increasing pH (Larson and Skold, 1958; Stumm, 1960; Hatch, 1969; Pisigan and Singley, 1987). Iron levels, however, were usually reported to decrease with increasing pH (Karalekas et al., 1983; Kashinkunti et al., 1999; Broo et al., 2001; Sarin et al., 2003). In a pipe loop system constructed from 90- to100-year-old unlined cast iron pipes taken from a Boston distribution system, iron concentrations were found to steadily decrease when the pH was raised from 7.6 to 9.5 (Sarin et al., 2003). Similarly, when iron was measured in the distribution system following a pH increase from 6.7 to 8.5, a consistent downward trend in iron concentrations was found over 2 years (Karalekas et al., 1983). These observations are consistent with the fact that the solubility of iron-based corrosion by-products decreases with increasing pH.

Water with low pH, low alkalinity and low calcium is particularly aggressive towards cement materials. The water quality problems that may occur are linked to the chemistry of the cement. Lime from the cement releases calcium ions and hydroxyl ions into the drinking water. This, in turn, may result in a substantial pH increase, depending on the buffering capacity of the water (Leroy et al., 1996). Pilot-scale tests were conducted to simulate low-flow conditions of newly lined cement mortar pipes carrying low-alkalinity water (Douglas et al., 1996). In the water with an initial pH of 7.2, alkalinity of 14 mg/L as calcium carbonate and calcium at 13 mg/L as calcium carbonate, measures of pH as high as 12.5 were found.

Similarly, in the water with an initial pH of 7.8, alkalinity of 71 mg/L as calcium carbonate and calcium at 39 mg/L as calcium carbonate, measures of pH as high as 12 were found.

Understanding Amperometric Titration

It appears that DPD colorimetric determination and amperometric titration as described in Standard Methods are the procedures most commonly used for routine measurement of total chlorine. Few studies have been conducted to evaluate these or other total residual chlorine measurement techniques. Bender5 studied approximately 10 test procedures and found that results using the DPD colorimetric procedure were consistently higher than those using amperometric titration. Brooks and Seegert6 described an amperometric titration procedure employing a recording polargraph and microburette, which was reported to be accurate and free from interference. The reliability of the DPD colorimetric method for free chlorine has been increasingly questioned in recent years. The suitability of that procedure for accurate total chlorine determinations appears to the authors to be questionable, as well. Amperometric titration as described in Standard Methods cannot be used to measure total chlorine concentrations less than about 0.05 mg/L, which is at least an order of magnitude greater than levels of concern in natural waters for potential toxicity to aquatic organisms. A reliable, simple procedure for low-level total chlorine determinations is clearly needed.

Analytical Procedure

Section 409C of Standard Methods includes a General Discussion section on amperometric titration for the determination of chlorine in aqueous solutions. That discussion is applicable to the procedure used by the authors. Also included in Standard Methods is a section concerning the titration apparatus. Basically, the titration equipment consists of a buret capable of accurately delivering 0.01 mL of titrant, a sample cup, and a stirring device in which is housed a platinum electrode and a KCl reference electrode. Several companies manufacture amperometric titrators that fit this general description. The experience of the senior author is that some of the commercial titrators are less suitable than others, primarily because of the small surface area of some of the electrodes employed. A Wallace and Tiernan amperometric titrator was used by the authors in developing and applying the procedure described below.

Reagents

a. Chlorine-free water. Only distilled or demineralized water that is free of chlorine should be used in preparing reagents. Chlorine-free water may be prepared by passing distilled or demineralized water through a suitable activated carbon filter adsorption column. The water may be tested for the presence of chlorine by titrating a sample as described in the Procedure section. Any deflection in the meter upon the addition of PAO titrant indicates the presence of chlorine or other oxidants that would interfere in the titration procedure.

b. Standard phenylarsine oxide (PAO), 0.00564 N. See Standard Methods Section 409B, paragraph 3a.

Standardization – Dilute 50.00 mL of freshly prepared 0.0002256 N potassium biniodate to 200 mL in chlorine-free water. Add approximately 1.5 g KI and stir to dissolve. Add 1 mL acetate buffer and allow to stand in the dark for 6 minutes. Titrate using the amperometric titrator and determine the equivalence point as detailed in the Procedure section. If the standard PAO is 0.00564 N, exactly 2.00 mL of PAO will be required to reach the equivalence point.

c. Phenylarsine oxide titrant, 0.000564 N. Dilute 10.00 mL of 0.00564 N PAO to 100.0 mL in chlorine-free water.

Standardization – Dilute 5.00 mL of 0.0002256 N potassium biniodate to 200 mL with chlorine-free water. Add approximately 1.5 g KI and stir to dissolve. Add 1 mL acetate buffer and allow to stand in the dark for 6 minutes. Titrate using the amperometric titrator and determine the equivalence point as detailed in the Procedure section below. If the PAO titrant is 0.000564 N, exactly 2.00 mL of PAO will be required to reach the equivalence point.

d. Potassium biniodate, 0.0002256 N. Dissolve 0.7332 g reagent grade $KH(IO_3)2$ in 500 mL chlorine-free water and dilute to 1.00 L. Dilute 10.00 mL of that solution to 100.0 mL with chlorine-free water. That solution is used for the standardization of the PAO and should be freshly prepared.

e. Acetate buffer solution, pH 4. See Standard Methods1 Section 409B, paragraph 3e.

f. Potassium iodide, (KI), reagent grade crystals.

Procedure

a. Titrant selection. Normally a 200-mL sample is used in titration. Each 0.1 mL of 0.000564 N PAO corresponds to 0.01 mg/L in a 200-mL sample. The titrant normality should be selected such that no more than about 4 mL of titrant will be required to reach the equivalence point. Thus, if the chlorine concentration in the majority of the samples to be titrated is less than about 0.4 mg/L, use 0.000564 N PAO as the titrant. If only samples containing chlorine concentrations in excess of 0.4 mg/L are to be analyzed, use 0.00564 N PAO as the titrant. If samples containing concentrations of chlorine in excess of about 0.4 mg/L are to be titrated only occasionally and the volume of 0.000564 N PAO required for titration is found to be excessive, a suitable subsample may be used and diluted to 200 mL with chlorine-free water.

b. Titration procedure (total residual chlorine). Prior to beginning the titration, rinse the buret with PAO titrant by filling it completely and allowing the titrant to run into an empty sample cup. Repeating this operation three or four times will ensure that the correct titrant concentration reaches the sample cup. Remove the sample cup and rinse with distilled water and with the sample to be titrated. Add 200 mL of the sample to the sample cup. Add approximately 1.5 g (± 0.2 g) crystalline KI and allow to dissolve, using the agitator on the titrator for mixing.

The exact amount of KI added is not critical, but the analyst should weigh 1.5 g of this reagent periodically to become familiar with the approximate amount required. Add 1 mL of acetate buffer and allow the microammeter on the titrator to reach a stable reading; the titration should be started within about 30 seconds following the addition of the KI to the sample.

Full-scale deflection on the microammeter is 100 units. The meter should be initially adjusted to read between 90 and 100 units. Record the initial reading prior to the addition of titrant. Titrate by adding suitable volumes of titrant and recording the titrant volume added and the resultant current reading. At least three (and preferably five to ten) readings of current and titrant volume added should be obtained prior to passing the equivalence point; then add excess titrant to ensure that there is no further meter deflection. Record the final meter reading. If, during the titration, the meter reading falls to near or below 10 units, record the low reading, re-adjust the meter to read between 90 and 100 units, record the high reading, and continue the titration. This approach allows calculation of the total meter deflection, which is used in determining the equivalence point.

The equivalence point is determined by plotting the total meter deflection as a function of titrant volume added. It is important that the total meter deflection be used in preparing this plot. A straight line is drawn through the first few points in the plot and a second straight line is drawn parallel to the abscissa and corresponding to the final total deflection in the meter reading. The equivalence point is determined by the intersection of those two lines. When 0.000564 N PAO is used as the titrant, the chlorine concentration is 0.1-times the titrant volume at the equivalence point. This plotting procedure is also outlined in the ASTM Water Manual8 under procedures ASTM D1253 (Tests for Residual Chlorine in Water) and ASTM D1427 (Tests for Residual Chlorine in Waste Water).

c. Sample storage and handling. Chlorine measurements should be made as soon after sample collection as possible. Samples to be analyzed for chlorine should be stored in the dark and packed on ice if they must be held for more than a few minutes before analysis. Chlorine compounds are highly reactive and may be rapidly lost from samples due to the effects of volatilization, phototransformation, and chlorine demand. Storage of samples on ice and in the dark between sampling and analysis will help minimize the rate of dissipation. It is important to estimate the changes that occur in chlorine content in the subject water between sample collection and analysis.

This can be accomplished by performing a "time-lag" test. To perform a time-lag test, a single large (approximately 2-L) sample of the water being analyzed is collected. The chlorine concentration in that sample is determined six to ten times over a period of one to three hours, depending on the normal sample holding time. The measured concentrations are then plotted as a function of time, normally on semilog paper. In most cases, the decrease in chlorine concentration over time can be described by first-order reaction kinetics.

The original chlorine content in any sample can be computed given the measured concentration and the holding time. A time-lag study should be performed on a regular basis for each type of water being analyzed because of variability in water compositions. The sample set used for the study should be handled in the same way as other samples (i.e., the samples should be kept cold and in the dark). Even when time-lag studies are made a part of the routine analytical procedure, it is important that the delay between sample collection and chlorine analysis be held to a minimum.

Sodium Hypochlorite
Sodium Hypochlorite, or bleach, is produced by adding elemental chlorine to sodium hydroxide. Typically, hypochlorite solutions contain from 5 to 15% chlorine, and are shipped by truck in one- to 5,000- gallon containers.

Advantages
- ✓ Solution is less hazardous and easier to handle than elemental chlorine
- ✓ Fewer training requirements and regulations than elemental chlorine

Limitations
- ✓ Limited shelf-life
- ✓ Potential to add inorganic byproducts (chlorate, chlorite and bromate) to water
- ✓ Corrosive to some materials and more difficult to store than most solution chemicals
- ✓ Higher chemical costs than elemental chlorine

Calcium Hypochlorite
- ✓ Calcium hypochlorite is another chlorinating chemical used primarily in smaller applications. It is a white, dry solid containing approximately 65% chlorine, and is commercially available in granular and tablet forms.

Advantages
- ✓ More stable than sodium hypochlorite, allowing longer storage
- ✓ Fewer training requirements and regulations than elemental chlorine

Limitations
- ✓ Dry chemical requires more handling than sodium hypochlorite
- ✓ Precipitated solids formed in solution complicate chemical feeding
- ✓ Higher chemical costs than elemental chlorine
- ✓ Fire or explosive hazard if handled improperly
- ✓ Potential to add inorganic byproducts (chlorate, chlorite and bromate) to water

Onsite Hypochlorite Generation

In recent years some municipalities have installed on-site hypochlorite generators that produce weak hypochlorite solutions (~0.8%) using an electrolytic cell and a solution of salt water.

Advantages

- ✓ Minimal chemical storage and transport

Limitations

- ✓ More complex and requires a higher level of maintenance and technical expertise
- ✓ High capital cost
- ✓ Operating costs are often higher than for commercial hypochlorite
- ✓ Requires careful control of salt quality
- ✓ Weak solution requires high volume chemical feed and control
- ✓ Byproducts in generated hypochlorite may be difficult to monitor and control
- ✓ System backup may be more difficult and costly

Ozone

Ozone (O_3) is generated on-site at water treatment facilities by passing dry oxygen or air through a system of high voltage electrodes. Ozone is one of the strongest oxidants and disinfectants available. Its high reactivity and low solubility, however, make it difficult to apply and control. Contact chambers are fully contained and non-absorbed ozone must be destroyed prior to release to avoid corrosive and toxic conditions. Ozone is more often applied for oxidation rather than disinfection purposes.

Advantages

- ✓ Strongest oxidant/disinfectant available
- ✓ Produces no chlorinated THMs, HAAs
- ✓ Effective against Cryptosporidium at higher concentrations
- ✓ Used with Advanced Oxidation processes to oxidize refractory organic compounds

Limitations

- ✓ Process operation and maintenance requires a high level of technical competence
- ✓ Provides no protective residual
- ✓ Forms brominated byproducts (bromate, brominated organics)
- ✓ Forms nonhalogenated byproducts (ketenes, organic acids, aldehydes)
- ✓ Breaks down more complex organic matter; smaller compounds can enhance microbial re-growth in distribution systems and increase DBP formation during secondary disinfection processes.
- ✓ Higher operating and capital costs than chlorination
- ✓ Difficult to control and monitor particularly under variable load conditions

Ultraviolet Radiation

Ultraviolet (UV) radiation, generated by mercury arc lamps, is a non-chemical disinfectant. When UV radiation penetrates the cell wall of an organism, it damages genetic material, and prevents the cell from reproducing. Although it has a limited track record in drinking water applications, UV has been shown to effectively inactivate many pathogens while forming limited disinfection byproducts.

Advantages

- ✓ Effective at inactivating most viruses, spores and cysts
- ✓ No chemical generation, storage, or handling
- ✓ Effective against Cryptosporidium
- ✓ No known byproducts at levels of concern

Limitations

- ✓ No residual protection
- ✓ Low inactivation of some viruses (reoviruses and rotaviruses)

- ✓ Difficult to monitor efficacy
- ✓ Irradiated organisms can sometimes repair and reverse the destructive effects of UV through a process known as photo-reactivation
- ✓ May require additional treatment steps to maintain high-clarity water
- ✓ Does not provide oxidation, or taste and odor control
- ✓ High cost of adding backup/emergency capacity
- ✓ Mercury lamps may pose a potable water and environmental toxicity risk

Alternative Disinfectants

Up until the late 1970s, chlorine was virtually the only disinfectant used to treat drinking water. Chlorine was considered an almost ideal disinfectant, based on its proven characteristics:

- ✓ Effective against most known pathogens
- ✓ Provides a residual to prevent microbial re-growth and protect treated water throughout the distribution system
- ✓ Suitable for a broad range of water quality conditions
- ✓ Easily monitored and controlled

Test methods available for Residual Chlorine

Residual Chlorine can be measured using different methods. Iodometric and DPD colorimetric methods are the most common methods. Each method has its own set of reagents and concentration range.

Iodometric Method
Residual Chlorine by Iodometric has a minimum detectable concentration of 40ppb if 0.01N sodium thiosulfate is used. Prepare the sample for titration by adding 5mL of acetic acid and 1g of potassium iodide to the sample. Titrate the sample with 0.01N sodium thiosulfate. Concentrations below 1 mg/L should be measured by using either 0.00564N sodium thiosulfate or 0.00564N phenylarsine oxide.

DPD Colorimetric Method
Residual Chlorine can also be measured by the DPD Colorimetric method. This method has a minimum detectable concentration of 10ppb. In this method, the calibration is either made up from a chlorine solution or a potassium permanganate solution. The typical calibration range for this method is 0.05 to 4mg/L. The reagents used in this method are a phosphate buffer and N,N-diethyl-p-phenylenediamine indicator solution. The samples are mixed with the reagents and then read on a spectrophotometer at a wavelength of 515nm.

Chlorine in water solutions is not stable. As a result, its concentration in samples decreases rapidly. Exposure to sunlight or other strong light, air, or agitation will further reduce the quantity of chlorine present in solutions. Samples to be analyzed for chlorine cannot be stored or preserved. Tests must be started immediately after sampling. Therefore, samples taken for the chlorine residual test must be grab samples only and excessive agitation must be avoided.

It is not necessary to use special sampling devices or containers for the chlorine residual test. However, the sampling container should be capable of collecting samples from a representative sampling point following chlorine contact, and should be made of resistant materials that will not rust or corrode, and which can be easily cleaned.

NOTE: A long handled aluminum dipper attached to a wooden handle, or an equivalent device, is acceptable for collecting samples. Do not use coffee cans, bleach bottles, etc.

Preparation of Chemicals
At a minimum, hand and eye protection should be used when handling any of the chemicals mentioned in this section. Before working with any chemical, consult the appropriate Material Safety Data Sheet (MSDS) to determine if other safety precautions are necessary.

Chlorine Residual Reagents
Iodometric and Amperometric Methods:
I. Standard Phenylarsine Oxide (PAO) Solution, 0.00564 N

A. Prepare 0.3 N sodium hydroxide solution (NaOH) by dissolving 12.0 g NaOH in 800 mL distilled water and diluting to 1 liter.

B. Prepare a 6.0 N hydrochloric acid solution (HCl) by adding 108 mL concentrated HCl to 800 mL distilled water and diluting to 1 liter. (Caution: Concentrated HCl fumes can burn eyes and lungs—do not breathe fumes!)

C. Prepare an approximately 0.00564 N solution of PAO using the following procedures:

1. Dissolve approximately 0.8 g PAO powder in 150 mL of 0.3 N NaOH solution, and allow to settle.

2. Decant 110 mL into 800 mL distilled water and mix thoroughly.

3. Bring to pH 6 to 7 with 6N HCl and dilute to 950 mL with distilled water. (Caution: PAO is poisonous. Wash thoroughly after use and do not ingest.)

D. Standardization

1. Accurately measure 5 to 10 mL freshly standardized 0.0282 N iodine solution into a flask and add 1 mL potassium iodide solution (50g KI dissolved and diluted to 1 L with freshly boiled and cooled distilled water.

2. Titrate with PAO solution, using starch solution as an indicator, until blue disappears.

3. Normality (N) of PAO = (mL iodine solution x 0.0282)/mL PAO titrated.

4. Adjust PAO to 0.00564 N and recheck.

II. Standard Sodium Thiosulfate Solution, 0.00564 N

A. Prepare a 0.1 N sodium thiosulfate solution by dissolving 25 g $Na_2S_2O_3$ $5H_2O$ in 1000 mL of freshly boiled distilled water. Store reagent for at least 2 weeks to allow oxidation of any bisulfite ion present. Add a few mL of chloroform ($CHCl_3$) to minimize bacterial decomposition.

Standardize by one of the following methods:

1. Iodate Method

a. Dissolve 3.249 g anhydrous primary standard quality potassium bi-iodate ($KH(IO_3)2$) or 3.567 g potassium iodate (KIO_3) dried at 103 +/-2°C for 1 hour in distilled water and dilute to 1000 mL to yield a 0.1000 N iodate solution. Store in a glass stoppered bottle.

b. Add, with constant stirring, 1 mL concentrated sulfuric acid (H_2SO_4), 10 mL 0.1000 N iodate solution, and 1 g potassium iodide (KI) to 80 mL distilled water. Titrate immediately with 0.1 N sodium thiosulfate ($Na_2S_2O_3$) until the yellow color of the liberated iodine is almost discharged. Add 1 mL starch indicator solution and continue titration until the blue color disappears.

c. The normality (N) of the sodium thiosulfate is calculated as follows: N of $Na_2S_2O_3$ = 1/mL $Na_2S_2O_3$ for titration

2. Dichromate Method

A. Dissolve 4.904 g anhydrous primary standard grade potassium dichromate ($K_2Cr_2O_7$) in distilled water and dilute to 1000 mL to yield a 0.1000 N dichromate solution. Store in a glass stoppered bottle.

B. For maximum stability of the standard 0.00564 N sodium thiosulfate solution, prepare by diluting an aged 0.1N $Na_2S_2O_3$ standard solution with freshly boiled distilled water. Add 10 mg Mercuric iodide and 4 g of sodium borate per liter of solution. Standardize daily using 0.00564 N potassium dichromate or iodate solution.

III. Standard Iodine Solution (I_2), 0.1 N

A. Dissolve 40 g potassium iodide (KI) in 25 mL chlorine-demand-free water.

B. Add 13 g resublimed iodine (I_2) and stir until dissolved.

C. Transfer to a 1 liter volumetric flask and dilute to the mark.

D. Standardization

1. Volumetrically measure 40 to 50 mL 0.1 N arsenite solution into a flask.

2. Titrate with 0.1 N iodine solution using starch solution as an indicator.

3. Just before end-point is reached, add a few drops of hydrochloric acid solution to liberate sufficient carbon dioxide (CO_2) to saturate the solution.

4. Titrate until blue color first appears and remains.

5. Normality (N) of iodine = (mL of arsenite solution used x 0.1)/mL of iodine titrated

IV. Standard Iodine Titrant (I_2), 0.0282 N

A. Dissolve 25 g KI in a bottle of distilled water in a 1L volumetric flask.

B. Add the correct amount of the exactly standardized 0.1 N iodine solution to yield a 0.0282 N solution.

C. Dilute to one liter with chlorine-demand-free water.

D. Store iodine solutions in amber bottles or in the dark, and protect from exposure to direct sunlight. Do not use rubber stoppers; keep iodine from all contact with rubber.

E. Check titrant normality daily against 0.00564 N PAO or sodium thiosulfate solution. A procedure for calculating a correction factor for this titrant is given in Appendix C.

V. Standard Potassium Iodate Titrant (KIO3), 0.00564 N

A. Dissolve 201.2 mg primary standard grade potassium iodate (KIO_3), dried for 1 hour at 103°C, or 183.3 mg primary standard grade anhydrous potassium bi-iodate ($KH(IO_3)2V$) in distilled water.

B. Dilute to 1 liter volumetrically.

C. Store in glass bottles in the dark and protect from exposure to direct sunlight.

VI. Potassium Iodide Solution (KI), 5% W/V

A. Dissolve 50 g KI in freshly boiled and cooled distilled water and dilute to 1 liter.

B. Store in a brown glass-stoppered bottle in the dark, preferably at 4°C.

C. Discard when solution becomes yellow.

VII. Acetate Buffer Solution, pH 4.0

A. Dissolve 146 g anhydrous sodium acetate ($NaC_2H_3O_2$ $3H_2O$) in 400 mL distilled water.

B. CAREFULLY add 458 mL concentrated (glacial) acetic acid.

C. Dilute to 1 liter with chlorine-demand-free water.

VIII. Standard Arsenite Solution (As_2O_3), 0.1N

A. Accurately weigh a dried, cooled stoppered weighing bottle.

NOTE: Use forceps or tongs—do not handle weighing bottle with fingers.

B. In weighing bottle, weigh out approximately 4.95 g arsenic trioxide (As_2O_3).

C. Transfer without loss to a 1 liter volumetric flask

NOTE: Do not attempt to brush out remaining arsenic trioxide).

D. Reweigh bottle and record weight of arsenic trioxide transferred.

E. Add enough distilled water to moisten the arsenic trioxide.

F. Add 15 g sodium hydroxide (NaOH) and 100 mL distilled water.

G. Swirl flask gently until As_2O_3 is dissolved.

H. Dilute to 250 mL and saturate the solution with carbon dioxide (CO_2) by bubbling CO_2 gas through the solution for a few minutes.

NOTE: This converts the sodium hydroxide (NaOH) to sodium bicarbonate ($NaHCO_3$).

I. Dilute to the 1 liter mark, stopper, and mix thoroughly.

J. This solution has an almost indefinite shelf life.

CAUTION: This solution is highly poisonous and is a suspected cancer causing agent: handle carefully!

IX. Starch Indicator

A. Weigh out 5 g soluble or potato starch.

B. Add enough distilled water to make a thin paste.

C. Pour into 1 liter boiling distilled water, stir and let settle overnight.

D. Transfer clear supernatant into a storage container and preserve by adding 1.25 g salicylic acid, 4 g zinc chloride, or a combination of 4 g sodium propionate and 2 g sodium azide per liter of starch solution.

E. Some commercial starch substitutes or powder indicators are acceptable.

X. Phosphoric Acid solution (H_3PO_4), 1 + 9

A. Carefully add 100 mL of phosphoric acid (H_3PO_4), 85%, to 900 mL of freshly boiled distilled water.

B. Caution should be used when handling this solution, as it can be corrosive.

XI. Phosphoric Acid—Sulfamic Acid Solution

A. Dissolve 20 g sulfamic acid (NH_2SO_3H) in 1 liter of 1 + 9 phosphoric acid (H_3PO_4).

DPD Titrimetric Method

I. Phosphate Buffer Solution

A. Dissolve 24 g anhydrous disodium hydrogen phosphate (Na_2HPO_4) in 400 to 500 mL distilled water.

B. Add 46 g anhydrous potassium dihydrogen phosphate (KH_2PO_4).

C. Dissolve 800 mg disodium ethylenediaminetetraacetate dihydrate (EDTA) in a separate container.

NOTE: This chemical is also known as (ethylenediamine) tetraacetic acid sodium salt.

D. Combine the 2 solutions and dilute to 1 liter.

E. Add 20 mg mercuric chloride to prevent mold growth.

F. Caution: Mercuric chloride is toxic. Take care to avoid ingestion.

II. DPD Indicator Solution

A. Add 8 mL of a 1 + 3 sulfuric acid solution (H_2SO_4) into 500 mL distilled water. Prepare by mixing one part concentrated H_2SO_4 to 3 parts distilled water. (For example, 5 mL H_2SO_4 to 15 mL distilled water.)

B. Add 200 mg EDTA (disodium ethylenediaminetetraacetate dihydrate).

C. Add 1 g DPD Oxalate (N, N-Diethyl-p-phenylenediamine oxalate).

D. Dilute to 1 liter and store in a brown glass-stoppered bottle and discard when discolored.

CAUTION: The DPD oxalate is poisonous, handle carefully!

III. Standard Ferrous Ammonium Sulfate (FAS) Titrant, 0.00282 N

A. Add 1 mL of 1 + 3 sulfuric acid solution (H2SO4) to 500 mL of freshly boiled and cooled distilled water. Prepare by adding one part concentrated H2SO4 to 3 parts distilled water.

B. Dissolve 1.106 g ferrous ammonium sulfate ($Fe(NH_4)2(SO_4)2\ 6H_2O$)

C. Dilute to 1 liter.

D. This standard can be used for 1 month before replacement.

E. Standardize weekly using the following procedure:

1. Measure 100 mL of FAS standard solution into an Erlenmeyer flask.

2. Add 10 mL of 1 + 5 sulfuric acid. Prepare by adding one part concentrated H_2SO_4 to 5 parts distilled water.

3. Add 5 mL concentrated phosphoric acid.

4. Add 2 mL 0.1% barium diphenylamine sulfonate indicator. Prepare by dissolving 0.1 g ($C_6H_5NHC_6H_4$-4-SO_3) Ba in 100 mL distilled water.

5. Titrate with 0.100N potassium dichromate (see iodometric and amperometric section for preparation directions) to a violet end-point that persists for 30 seconds.

DPD Colorimetric Method

I. Phosphate Buffer Solution

(see DPD Titrimetric Method chemicals)

II. DPD Indicator Solution

(see DPD Titrimetric Method chemicals)

III. Potassium Permanganate Stack Solution

A. Dissolve 891 mg potassium permanganate ($KMnO_4$) in distilled water and dilute to 1000 mL.

IV. Potassium Permanganate Standard Solution

A. Dilute 10 mL of stock solution to 100 mL in a volumetric flask.

B. 1 mL of the standard solution diluted to 100 mL with distilled water will be equivalent to 1.0 mg/L chlorine residual in a DPD reaction.

C. Prepare standard solutions by diluting appropriate volumes to 100 mL with distilled water.

If a direct concentration readout colorimeter is used, the DPD and buffer reagents should be prepared or ordered in accordance with the instrument manufacturer's instructions. If the Hach DR100 colorimeter is used, the prepared DPD powder pillows used with the Hach direct reading colorimeters may be purchased from the Hach Company at the following address:

Hach Company
P.O. Box 389
Loveland, Colorado 80539

Orion Model 97-70 Electrode Method

With the exception of the 1 ppm potassium iodate standard and the chlorine water (100 ppm), all of the reagents required for this method can be purchased from Orion Research at the following address:

Orion Research Incorporated
840 Memorial Drive
Cambridge, Massachusetts 02139

I. Prepare a 1 mg/L iodate standard by volumetrically diluting 1 mL of the 100 ppm iodate standard to 100 mL with distilled water.

II. Prepare the chlorine water (approximately 100 ppm) by diluting 1 mL hypochlorite solution (household chlorine bleach) to 500 mL with distilled water.

Hach Model CN-66 Test Kit Method
The DPD indicator powder pillows used in the Hach Model CN-66 Test Kit may be purchased from the Hach Company at the following address:

Hach Company
P.O. Box 389
Loveland, Colorado 80539

Microscopic Waterborne Agents

It is easy to take for granted the safety of modern municipal drinking water, but prior to widespread filtration and chlorination, contaminated drinking water presented a significant public health risk. The microscopic waterborne agents of cholera, typhoid fever, dysentery and hepatitis A killed thousands of U.S. residents annually before disinfection methods were employed routinely, starting about a century ago. Although these pathogens are defeated regularly now by technologies such as chlorination, they should be thought of as ever-ready to stage a come-back given conditions of inadequate or no disinfection.

Understanding Bacteria
Bacteria are microorganisms often composed of single cells shaped like rods, spheres or spiral structures. Prior to widespread chlorination of drinking water, bacteria like Vibrio cholerae, Salmonella typhii and several species of Shigella routinely inflicted serious diseases such as cholera, typhoid fever and bacillary dysentery, respectively. As recently as 2000, a drinking water outbreak of E. coli in Walkerton, Ontario sickened 2,300 residents and killed seven when operators failed to properly disinfect the municipal water supply.
While developed nations have largely conquered water-borne bacterial pathogens through the use of chlorine and other disinfectants, the developing world still grapples with these public health enemies

Understanding Viruses
Viruses are infectious agents that can reproduce only within living host cells. Shaped like rods, spheres or filaments, viruses are so small that they pass through filters that retain bacteria. Enteric viruses, such as hepatitis A, Norwalk virus and rotavirus are excreted in the feces of infected individuals and may contaminate water intended for drinking. Enteric viruses infect the gastrointestinal or respiratory tracts, and are capable of causing a wide range of illness, including diarrhea, fever, hepatitis, paralysis, meningitis and heart disease (American Water Works Association, 1999).

Understanding Protozoan Parasites
Protozoan parasites are single-celled microorganisms that feed on bacteria found in multicellular organisms, such as animals and humans. Several species of protozoan parasites are transmitted through water in dormant, resistant forms, known as cysts and oocysts. According to the World Health Organization, Cryptosporidium parvum oocysts and Giardia lamblia cysts are introduced to waters all over the world by fecal pollution. The same durable form that permits them to persist in surface waters makes these microorganisms resistant to normal drinking water chlorination (WHO, 2002b). Water systems that filter raw water may successfully remove protozoan parasites.

Emerging Pathogens
An emerging pathogen is one that gains attention because it is one of the following:

- a newly recognized disease-causing organism
- a known organism that starts to cause disease
- an organism whose transmission has increased

Understanding Oxidizing Agents
Oxidizing agents act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death. A large number of disinfectants operate in this way. Chlorine and oxygen are strong oxidizers, so their compounds figure heavily here.

- ✓ Sodium hypochlorite is very commonly used. Common household bleach is a sodium hypochlorite solution and is used in the home to disinfect drains, toilets, and other surfaces. In more dilute form, it is used in swimming pools, and in still more dilute form, it is used in drinking water. When pools and drinking water are said to be chlorinated, it is actually sodium hypochlorite or a related compound—not pure chlorine—that is being

used. Chlorine partly reacts with proteinaceous liquids such as blood to form non-oxidizing N-chloro compounds, and thus higher concentrations must be used if disinfecting surfaces after blood spills. Commercial solutions with higher concentrations contain substantial amounts of sodium hydroxide for stabilization of the concentrated hypochlorite, which would otherwise decompose to chlorine, but the solutions are strongly basic as a result.

- ✓ Other hypochlorites such as calcium hypochlorite are also used, especially as a swimming pool additive. Hypochlorites yield an aqueous solution of hypochlorous acid that is the true disinfectant. Hypobromite solutions are also sometimes used.
- ✓ Electrolyzed water or "Anolyte" is an oxidizing, acidic hypochlorite solution made by electrolysis of sodium chloride into sodium hypochlorite and hypochlorous acid. Anolyte has an oxidation-reduction potential of +600 to +1200 mV and a typical pH range of 3.5—8.5, but the most potent solution is produced at a controlled pH 5.0–6.3 where the predominant oxychlorine species is hypochlorous acid.
- ✓ Chloramine is often used in drinking water treatment.
- ✓ Chloramine-T is antibacterial even after the chlorine has been spent, since the parent compound is a sulfonamide antibiotic.
- ✓ Chlorine dioxide is used as an advanced disinfectant for drinking water to reduce waterborne diseases. In certain parts of the world, it has largely replaced chlorine because it forms fewer byproducts. Sodium chlorite, sodium chlorate, and potassium chlorate are used as precursors for generating chlorine dioxide.
- ✓ Hydrogen peroxide is used in hospitals to disinfect surfaces and it is used in solution alone or in combination with other chemicals as a high level disinfectant. Hydrogen peroxide is sometimes mixed with colloidal silver. It is often preferred because it causes far fewer allergic reactions than alternative disinfectants. Also used in the food packaging industry to disinfect foil containers. A 3% solution is also used as an antiseptic.
- ✓ Hydrogen peroxide vapor is used as a medical sterilant and as room disinfectant. Hydrogen peroxide has the advantage that it decomposes to form oxygen and water thus leaving no long term residues, but hydrogen peroxide as with most other strong oxidants is hazardous, and solutions are a primary irritant. The vapor is hazardous to the respiratory system and eyes and consequently the OSHA permissible exposure limit is 1 ppm (29 CFR 1910.1000 Table Z-1) calculated as an eight hour time weighted average and the NIOSH immediately dangerous to life and health limit is 75 ppm. Therefore, engineering controls, personal protective equipment, gas monitoring etc. should be employed where high concentrations of hydrogen peroxide are used in the workplace. Vaporized hydrogen peroxide is one of the chemicals approved for decontamination of anthrax spores from contaminated buildings, such as occurred during the 2001 anthrax attacks in the U.S. It has also been shown to be effective in removing exotic animal viruses, such as avian influenza and Newcastle disease from equipment and surfaces.
- ✓ The antimicrobial action of hydrogen peroxide can be enhanced by surfactants and organic acids. The resulting chemistry is known as Accelerated Hydrogen Peroxide and is produced by Virox Technologies Inc. A 2% solution, stabilized for extended use, achieves high-level disinfection in 5 minutes, and is suitable for disinfecting medical equipment made from hard plastic, such as in endoscopes.[19] The evidence available suggests that products based on Accelerated Hydrogen Peroxide, apart from being good germicides, are safer for humans and benign to the environment.
- ✓ Iodine is usually dissolved in an organic solvent or as Lugol's iodine solution. It is used in the poultry industry. It is added to the birds' drinking water. In human and veterinary medicine, iodine products are widely used to prepare incision sites prior to surgery. Although it increases both scar tissue formation and healing time, tincture of iodine is used as an antiseptic for skin cuts and scrapes, and remains among the most effective antiseptics known.
- ✓ Ozone is a gas used for disinfecting water, laundry, foods, air, and surfaces. It is chemically aggressive and destroys many organic compounds, resulting in rapid decolorization and deodorization in addition to disinfection. Ozone decomposes relatively quickly, however, so that tap water chlorination cannot be entirely replaced by ozonation,

as the ozone would decompose already in the water piping. Instead, it is used to remove the bulk of oxidizable matter from the water, which would produce small amounts of organochlorides if treated with chlorine only.

- ✓ Peracetic acid is a disinfectant produced by reacting hydrogen peroxide with acetic acid. It is broadly effective against microorganisms and is not deactivated by catalase and peroxidase, the enzymes that break down hydrogen peroxide. It also breaks down to food safe and environmentally friendly residues (acetic acid and hydrogen peroxide), and therefore can be used in non-rinse applications. It can be used over a wide temperature range (0-40°C), wide pH range (3.0-7.5), in clean-in-place (CIP) processes, in hard water conditions, and is not affected by protein residues.
- ✓ Performic acid is the simplest and most powerful perorganic acid. Formed from the reaction of hydrogen peroxide and formic acid, it reacts more rapidly and powerfully than peracetic acid before breaking down to water and carbon dioxide.
- ✓ Potassium permanganate ($KMnO_4$) is a purplish-black crystalline powder that colors everything it touches, through a strong oxidizing action. This includes staining "stainless" steel, which somehow limits its use and makes it necessary to use plastic or glass containers. It is used to disinfect aquariums and is also widely used in community swimming pools to disinfect ones feet before entering the pool. Typically, a large shallow basin of KMnO4/water solution is kept near the pool ladder. Participants are required to step in the basin and then go into the pool. Additionally, it is widely used to disinfect community water ponds and wells in tropical countries, as well as to disinfect the mouth before pulling out teeth. It can be applied to wounds in dilute solution.

Understanding Waterborne Viruses

More than 100 types of human pathogenic viruses may be present in fecal-contaminated waters (Havelaar and others, 1993). Treatment processes and watershed management strategies designed on the basis of bacteriological criteria do not necessarily protect against viral infection because viruses are generally more persistent in the environment and are not removed as completely by treatment. In addition, because of their smaller size, viruses (0.023 to 0.080 µm) are transported further in ground water than bacteria (0.5 to 3µ m) or protozoan pathogens (4 to 15 µm) (Abbaszadegan and others, 1998). Because of the importance of viruses as a major public health concern, new methods for detection of enteric viruses and the search for indicators of viral contamination continue.

The current method for culturing enteric viruses under the ICR (U.S. Environmental Protection Agency, 1996c) is recognized as being difficult to implement; therefore, the ICR does not preclude the use of additional methods for research purposes. In addition, cell-culture methods are not available or suitable for all viruses of public health concern. One method, reverse-transcriptase-polymerase chain reaction (RT-PCR), a gene-probe method that amplifies and recognizes the nucleic acids of target viruses, has been adequately validated by the USEPA (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997) and is becoming widely used for environmental monitoring of enteric viruses. The RTPCR method, however, does not determine the infectivity of the virus, and it is technically demanding, time consuming, and costly for routine use.

Because monitoring of enteric viruses is recognized as being difficult and time consuming, some researchers advocate the use of coliphage as indicator viruses for fecal contamination (Sobsey and others, 1995). Coliphages are bacteriophages that infect and replicate in coliform bacteria. The two main groups of coliphages that are considered as candidates for viral indicators are somatic and F-specific coliphages.

Somatic coliphages infect coliform bacteria by attachment to the outer cell membrane or cell wall. They are widely distributed in both fecal-contaminated and uncontaminated waters; therefore, they may not be reliable indicators of fecal contamination (Sobsey and others, 1995). F-specific coliphages attach only to the F-pilus of coliforms that carry the F+ plasmid; F-pili are made only by bacteria grown at higher temperatures.

Hence, F-specific coliphages found in environmental samples presumably come from warm-blooded animals or sewage (Handzel and others 1993). Although somatic and F-specific coliphages are not consistently found in feces, they are found in high numbers in sewage and are thought to be reliable indicators of the sewage contamination of waters (International Association of Water Pollution Research and Control, 1991). Coliphage is also recognized to be representative of the survival and transport of viruses in the environment. To date, however, coliphage has not been found to correlate with the presence of pathogenic viruses.

Sampling Procedures

Streamwater Sample Collection

When designing a sampling plan, consider that the spatial and temporal distribution of microorganisms in surface water can be as variable as the distribution of suspended sediment because microorganisms are commonly associated with solid particles. The standard samplers used in by the majority of samplers can be used to collect streamwater samples for bacterial and viral indicators, Cryptosporidium, and Giardia providing that the equipment coming in contact with the water is properly cleaned and sterilized. For streamwater samples, these include the US-D77TM, US-D95, US-DH81, and weighted- and open-bottle samplers with autoclavable Teflon, glass, or polypropylene components.

• Prepare a separate set of sterile equipment (bottles nozzles, and caps) for sampling at each site.

• Follow sampling techniques given in Shelton (1994) to ensure that a sample is representative of the flow in the cross section. Use equal-width increment (EWI) or equal-discharge-increment (EDI) methods described in Edwards and Glysson (1988), unless site characteristics dictate otherwise.
• Because churn and cone splitters cannot be autoclaved, use a sterile 3-L bottle to composite subsamples for bacterial and viral indicators when using EDI and EWI methods. If possible, composite
by collecting subsamples at vertical locations in the cross section without overfilling the bottle.
• Alternatively, if the stream depth and (or) velocity is not sufficient to use depth-width integrating techniques, collect a sample by a hand-dip method (Myers and Sylvester, 1997).
• Collect approximately 1 L of streamwater for bacterial and viral indicators. Process the sample for E. coli and enterococci; send the remainder (at least 500 mL) on ice to the laboratory for C. perfringens and coliphage analysis.

Cryptosporidium and Giardia Analysis
For Cryptosporidium and Giardia analysis by Method 1623 (U.S. Environmental Protection Agency, 1999c), collect 10 L of streamwater for each protozoan pathogen using standard sampling techniques described in Myers and Sylvester (1997). Special sterilization procedures are needed for equipment used in the collection of samples for Cryptosporidium and Giardia. Autoclaving is not effective in neutralizing the epitopes on the surfaces of the oocysts and cysts that will react with the antibodies used for detection.
• Wash and scrub the equipment with soap and warm tap water to remove larger particulates and rinse with deionized water. Submerge the equipment in a vessel containing 12 percent hypochlorite solution for 30 minutes. Wash the equipment free of residual sodium hypochlorite solution with three rinses of filter-sterilized water; do not de-chlorinate the equipment using sodium thiosulfate. This procedure is best done in the office with dedicated sampling equipment for each site; however, it may be done in the field as long as the hypochlorite solution is stored and disposed of properly.
• Composite the sample in a 10-L cubitainer that is pre-sterilized by the manufacturer. The cubitainer is sent in a cardboard box to laboratory for Cryptosporidium analysis. The sample does not have to be kept on ice during transport. At this time, two methods are recommended for analysis of water samples for enteric viruses: (1) the reverse-transcriptase, polymerase chain reaction (RTPCR) method (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997) and (2) the cell-culture method (U.S. Environmental Protection Agency, 1996c). Sampling and equipment cleaning procedures are more thoroughly described elsewhere (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c). Briefly, 100 L of streamwater is pumped by means of a specially designed sampling apparatus and passed through a Virosorb1 1MDS filter (Cuno, Meriden, Conn.).The 1MDS filters, which remove viruses present in the water by charge interactions, are kept on ice and sent to a central laboratory for virus elution, concentration, and detection.

Ground-Water Sample Collection
Collecting
Ground-water samples by use of sterile techniques requires knowledge of the type of well, its use, its construction, and its condition.
• Swab the electronic tape used for water-level measurements with isopropyl or ethyl alcohol.
• In sampling subunit survey wells, once purging criteria have been met as described in Koterba and others (1995), collect the sample directly from the tap into a sterile container.
• Remove screens, filters, other devices from the tap before collecting the sample, and do not sample from leaking taps.

Because we are interested in the microbial population in the ground water and not in the distribution system, it is best to sample directly from the wellhead using a pump with sterile tubing, if possible.

Disinfection of Wastewater
The disinfection of potable water and wastewater provides a degree of protection from contact with pathogenic organisms including those causing cholera, polio, typhoid, hepatitis and a number of other bacterial, viral and parasitic diseases. Disinfection is a process where a significant percentage of pathogenic organisms are killed or controlled. As an individual pathogenic organism can be difficult to detect in a large volume of water or wastewater, disinfection efficacy is most often measured using "indicator organisms" that coexist in high quantities where pathogens are present. The most common indicator organism used in the evaluation of drinking water is Total Coliform (TC), unless there is a reason to focus on a specific pathogen.

The most common indicator organism for wastewater evaluation is fecal coliform but there has been discussion regarding the use of Escherichia coli (E. coli) or Total Coliform. As domestic wastewater contains approximately 1,000 times more indicator organisms than typical surface water, understanding wastewater disinfection will make it easier to understand water disinfection.

Chlorine gas is primarily a respiratory irritant and concentrations in air above one ppm can usually be detected by most persons. Chlorine causes varying degrees of irritation of the skin, mucus membranes, and the respiratory system, depending on the concentration and the duration of exposure. Severe exposure can cause death, but the severe irritating effect makes it unlikely that anyone would remain in the chlorine-containing atmosphere unless trapped or unconscious.

Liquid chlorine may cause skin and eye burns upon contact with these tissues. Chlorine produces no known cumulative or chronic effect, and complete recovery usually can be expected to occur shortly following mild, short term exposure. An eight-hour time-weighted exposure of one ppm and a one-hour weighted exposure are the current federal Occupational Safety and Health Administration (OSHA) standards.

Understanding Bacteriophage
Bacteriophages may have a lytic cycle or a lysogenic cycle, and a few viruses are capable of carrying out both. With lytic phages such as the T4 phage, bacterial cells are broken open (lysed) and destroyed after immediate replication of the virion. As soon as the cell is destroyed, the phage progeny can find new hosts to infect. Lytic phages are more suitable for phage therapy. Some lytic phages undergo a phenomenon known as lysis inhibition, where completed phage progeny will not immediately lyse out of the cell if extracellular phage concentrations are high. This mechanism is not identical to that of temperate phage going dormant and is usually temporary.

In contrast, the lysogenic cycle does not result in immediate lysing of the host cell. Those phages able to undergo lysogeny are known as temperate phages. Their viral genome will integrate with host DNA and replicate along with it fairly harmlessly, or may even become established as a plasmid. The virus remains dormant until host conditions deteriorate, perhaps due to depletion of nutrients; then, the endogenous phages (known as prophages) become active. At this point they initiate the reproductive cycle, resulting in lysis of the host cell. As the lysogenic cycle allows the host cell to continue to survive and reproduce, the virus is reproduced in all of the cell's offspring. An example of a bacteriophage known to follow the lysogenic cycle and the lytic cycle is the phage lambda of E. coli.

Sometimes prophages may provide benefits to the host bacterium while they are dormant by adding new functions to the bacterial genome in a phenomenon called lysogenic conversion. An eminent example is the conversion of a harmless strain of Vibrio cholerae by a phage into a highly virulent one, which causes cholera.

Attachment and Penetration
To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella. This specificity means a bacteriophage can infect only certain bacteria bearing receptors to which they can bind, which in turn determines the phage's host range. Host growth conditions also influence the ability of the phage to attach and invade them. As phage virions do not move independently, they must rely on random encounters with the right receptors when in solution (blood, lymphatic circulation, irrigation, soil water, etc.).

Myovirus bacteriophages use a hypodermic syringe-like motion to inject their genetic material into the cell. After making contact with the appropriate receptor, the tail fibers flex to bring the base plate closer to the surface of the cell; this is known as reversible binding. Once attached completely, irreversible binding is initiated and the tail contracts, possibly with the help of ATP present in the tail, injecting genetic material through the bacterial membrane. Podoviruses lack an elongated tail sheath similar to that of a myovirus, so they instead use their small, tooth-like tail fibers to enzymatically degrade a portion of the cell membrane before inserting their genetic material.

Virions
A virion is a complete functional virus that has the capacity to infect living tissue. This means that it includes the genetic material, the capsid, the enveloppe and the membrane proteins that allow the virus to bind to its host and enter it. A virus will not have an enveloppe within a cell. If the cell was burst artificially, then these virus particles cannot be called virion because they will lack certain proteins that will make them infectious even though the genetic material is present. Not all viruses have enveloppes, but most viruses have certain proteins that are necessary to permit them to enter the host cell.

Biomolecules found in virions: genetic material, either DNA or RNA, single or double stranded, nucleoprotein capsid, maybe an enveloppe usually receptor proteins or enzymes that permit binding or entry into the host. A viroid is a plant pathogen consisting of a circular piece of RNA without a protein coat.

Understanding Wastewater Disinfection

Wastewater Disinfection
There are a number of chemicals and processes that will disinfect wastewater, but none are universally applicable. Most septic tanks discharge into various types of subsurface wastewater infiltration systems (SWIS), such as tile fields or leach fields. These applications rely on the formation of a biomat at the gravel-soil interface where "biodegradation and filtration combine to limit the travel of pathogens." Aerobic treatment processes reduce pathogens, but not enough to qualify as a disinfection process. "Chlorination/dechlorination has been the most widely used disinfection technology in the U.S.; ozonation and UV light are emerging technologies." Each of these three methods have different considerations for the disinfection of wastewater.

Water Disinfection
Disinfection is usually the final stage in the water treatment process in order to limit the effects of organic material, suspended solids and other contaminants. Like the disinfection of wastewater, the primary methods used for the disinfection of water in very small (25-500 people) and small (501-3,300 people) treatment systems are ozone, ultraviolet irradiation (UV) and chlorine. There are numerous alternative disinfection processes that have been less widely used in small and very small water treatment systems, including chlorine dioxide, potassium permanganate, chloramines and peroxone (ozone/hydrogen peroxide).

Surface waters have been the focal point of water disinfection regulations since their inception, as groundwaters (like wells) have been historically considered to be free of microbiological contamination. Current data indicates this to not be true. Amendments to the Safe Drinking Water Act in 1996 mandate the development of regulations to require disinfection of groundwater "as necessary." While these regulations will apply to very small systems serving twenty-five people at least 60 days out of the year, the rules will not apply to private wells. However, the EPA recommends that wells be tested at least once per year and disinfected as necessary. While these proposed regulations have not yet been finalized, they will likely include; testing by each state, identification of contaminated water supplies, corrective action requiring disinfection and compliance monitoring. The rules are currently scheduled to be implemented in July 2003.

Residual Disinfection
The EPA requires a residual level of disinfection of water in pipelines to prevent microbial re-growth and help protect treated water throughout the distribution system. EPA”s maximum residual disinfection levels (MRDLs) are 4 mg/l for chlorine, 4 mg/l for chloramines and 0.8 mg/l for chlorine dioxide. Although chlorine levels are usually significantly lower in tap water, EPA believes that levels as high as the MRDLs pose no risk of adverse health effects, allowing for an adequate margin of safety (U.S. EPA, 1998a).

Chlorate Ion
The chlorate anion has the formula ClO-3. In this case, the chlorine atom is in the +5 oxidation state. "Chlorate" can also refer to chemical compounds containing this anion; chlorates are the salts of chloric acid. "Chlorate", when followed by a roman numeral in parentheses, e.g. chlorate (VII), refers to a particular oxyanion of chlorine. As predicted by VSEPR, chlorate anions have trigonal pyramidal structures.

Chlorates are powerful oxidizers and should be kept away from organics or easily oxidized materials. Mixtures of chlorate salts with virtually any combustible material (sugar, sawdust, charcoal, organic solvents, metals, etc.) will readily deflagrate. Chlorates were once widely used in pyrotechnics for this reason, though their use has fallen due to their instability. Most pyrotechnic applications which formerly used chlorates in the past now use the more stable perchlorates instead

Examples of chlorates include

- ✓ potassium chlorate, $KClO_3$
- ✓ sodium chlorate, $NaClO_3$
- ✓ magnesium chlorate, $Mg(ClO_3)2$

Chloride Ion

The chloride ion is formed when the element chlorine, a halogen, gains an electron to form an anion (negatively-charged ion) Cl-. The salts of hydrochloric acid contain chloride ions and can also be called chlorides. The chloride ion, and its salts such as sodium chloride, are very soluble in water. It is an essential electrolyte located in all body fluids responsible for maintaining acid/base balance, transmitting nerve impulses and regulating fluid in and out of cells.

The word chloride can also form part of the name of chemical compounds in which one or more chlorine atoms are covalently bonded. For example, methyl chloride, more commonly called chloromethane, (CH_3Cl) is an organic covalently bonded compound, which does not contain a chloride ion.

Chloride is used to form salts that can preserve food such as sodium chloride. Other salts such as calcium chloride, magnesium chloride, potassium chloride have varied uses ranging from medical treatments to cement formation.

An example is table salt, which is sodium chloride with the chemical formula NaCl. In water, it dissociates into Na+ and Cl- ions.

Examples of inorganic covalently bonded chlorides that are used as reactants are:

- ✓ Phosphorus trichloride, phosphorus pentachloride, and thionyl chloride, all three of which reactive chlorinating reagents that have been used in a laboratory.
- ✓ Disulfur dichloride (S_2Cl_2), used for vulcanization of rubber.

A chloride ion is also the prosthetic group present in the amylase enzyme. Another example is calcium chloride with the chemical formula $CaCl_2$. Calcium chloride is a salt that is marketed in pellet form for removing dampness from rooms. Calcium chloride is also used for maintaining unpaved roads and for sanite fortifying roadbases for new construction. In addition, Calcium chloride is widely used as a deicer since it is effective in lowering the melting point when applied to ice.

In the petroleum industry, the chlorides are a closely monitored constituent of the mud system. An increase of the chlorides in the mud system may be an indication of drilling into a high-pressure saltwater formation. Its increase can also indicate the poor quality of a target sand. Chloride is also a useful and reliable chemical indicator of river / groundwater fecal contamination, as chloride is a non-reactive solute and ubiquitous to sewage & potable water. Many water regulating companies around the world utilize chloride to check the contamination levels of the rivers and potable water sources.

Chlorite Ion

The chlorite ion is ClO_2-. A chlorite (compound) is a compound that contains this group, with chlorine in oxidation state +3. Chlorites are also known as salts of chlorous acid. Chlorine can assume oxidation states of -1, +1, +3, +5, or +7 within the corresponding anions Cl-, ClO-, ClO_2-, ClO_3-, or ClO_4-, known commonly and respectively as chloride, hypochlorite, chlorite, chlorate, and perchlorate. An additional oxidation state of +4 is seen in the neutral compound chlorine dioxide ClO_2, which has a similar structure to chlorite ClO_2- (oxidation state +3) and the cation chloryl (ClO_2+) (oxidation state +5).

Chlorine Dioxide

Chlorine dioxide is a chemical compound with the formula ClO_2. This yellowish-green gas crystallizes as bright orange crystals at -59 °C. As one of several oxides of chlorine, it is a potent and useful oxidizing agent used in water treatment and in bleaching. The molecule ClO2 has an odd number of valence electrons and it is therefore a paramagnetic radical. Its electronic structure has long baffled chemists because none of the possible Lewis structures are very satisfactory. In 1933 L.O. Brockway proposed a structure that involved a three-electron bond.

Chemist Linus Pauling further developed this idea and arrived at two resonance structures involving a double bond on one side and a single bond plus three-electron bond on the other. In Pauling's view the latter combination should represent a bond that is slightly weaker than the double bond. In molecular orbital theory this idea is commonplace if the third electron is placed in an anti-bonding orbital. Later work has confirmed that the HOMO is indeed an incompletely-filled orbital.

Chlorine dioxide is a highly endothermic compound that can decompose extremely violently when separated from diluting substances. As a result, preparation methods that involve producing solutions of it without going through a gas phase stage are often preferred. Arranging handling in a safe manner is essential.

In the laboratory, ClO_2 is prepared by oxidation of sodium chlorite:

$$2\ NaClO_2 + Cl_2 - 2\ ClO_2 + 2\ NaCl$$

Over 95% of the chlorine dioxide produced in the world today is made from sodium chlorate and is used for pulp bleaching. It is produced with high efficiency by reducing sodium chlorate in a strong acid solution with a suitable reducing agent such as methanol, hydrogen peroxide, hydrochloric acid or sulfur dioxide. Modern technologies are based on methanol or hydrogen peroxide, as these chemistries allows the best economy and do not co-produce elemental chlorine. The overall reaction can be written;

Chlorate + Acid + reducing agent - Chlorine Dioxide + By-products

The reaction of sodium chlorate with hydrochloric acid in a single reactor is believed to proceed via the following pathway:

$$HClO_3 + HCl - HClO_2 + HOCl$$

$$HClO_3 + HClO_2 - 2\ ClO_2 + Cl_2 + 2\ H_2O$$

$$HOCl + HCl - Cl_2 + H_2O$$

The commercially more important production route uses methanol as the reducing agent and sulfuric acid for the acidity. Two advantages by not using the chloride-based processes are that there is no formation of elemental chlorine, and that sodium sulfate, a valuable chemical for the pulp mill, is a side-product. These methanol-based processes provide high efficiency and can be made very safe.

A much smaller, but important, market for chlorine dioxide is for use as a disinfectant. Since 1999 a growing proportion of the chlorine dioxide made globally for water treatment and other small-scale applications has been made using the chlorate, hydrogen peroxide and sulfuric acid method, which can produce a chlorine-free product at high efficiency.

Traditionally, chlorine dioxide for disinfection applications has been made by one of three methods using sodium chlorite or the sodium chlorite - hypochlorite method:

$2\ NaClO_2 + 2\ HCl + NaOCl - 2\ ClO_2 + 3\ NaCl + H_2O$

or the sodium chlorite - hydrochloric acid method:

$5\ NaClO_2 + 4\ HCl - 5\ NaCl + 4\ ClO_2 + 2\ H_2O$

All three sodium chlorite chemistries can produce chlorine dioxide with high chlorite conversion yield, but unlike the other processes the chlorite-HCl method produces completely chlorine-free chlorine dioxide but suffers from the requirement of 25% more chlorite to produce an equivalent amount of chlorine dioxide. Alternatively, hydrogen peroxide may efficiently be used also in small scale applications.

Very pure chlorine dioxide can also be produced by electrolysis of a chlorite solution:

$2\ NaClO_2 + 2\ H_2O - 2\ ClO_2 + 2\ NaOH + H_2$

High purity chlorine dioxide gas (7.7% in air or nitrogen) can be produced by the Gas: Solid method, which reacts dilute chlorine gas with solid sodium chlorite.

$2\ NaClO_2 + Cl_2 - 2\ ClO_2 + 2\ NaCl$

These processes and several slight variations have been reviewed.

Haloacetic Acids
Haloacetic acids are carboxylic acids in which a halogen atom takes the place of a hydrogen atom in acetic acid. Thus, in a monohaloacetic acid, a single halogen would replace a hydrogen atom. For example, chloroacetic acid would have the structural formula CH_2ClCO_2H. In the same manner, in dichloroacetic acid two chlorine atoms would take the place of two hydrogen atoms ($CHCl_2CO_2H$). The inductive effect caused by the electronegative halogens often result in the higher acidity of these compounds by stabilizing the negative charge of the conjugate base.

Contaminants in Drinking Water
Haloacetic acids (HAAs) are a common undesirable by-product of drinking water chlorination. Exposure to such disinfection by-products in drinking water has been associated with a number of health outcomes by epidemiological studies, although the putative agent in such studies has not been identified.

In water, HAAs are stable, with the five most common being:

- ✓ monochloroacetic acid (MCA) $ClCH_2COOH$;
- ✓ dichloroacetic acid (DCA) $Cl_2CHCOOH$;
- ✓ trichloroacetic acid (TCA) Cl_3CCOOH;
- ✓ monobromoacetic acid (MBA) $BrCH_2COOH$;
- ✓ dibromoacetic acid (DBA) $Br_2CHCOOH$.

Collectively, these are referred to as the HAA5. HAAs can be formed by chlorination, ozonation or chloramination of water with formation promoted by slightly acidic water, high organic matter content and elevated temperature. Chlorine from the water disinfection process can react with organic matter and small amounts of bromide present in water to produce various HAAs. A study published in August 2006 found that total levels of HAAs in drinking water were not affected by storage or boiling, but that filtration was effective in decreasing levels.

Hypochlorites
Hypochlorites are calcium or sodium salts of hypochlorous acid and are supplied either dry or in liquid form (as, for instance, in commercial bleach). The same residuals are obtained as with gas chlorine, but the effect on the pH of the treated water is different. Hypochlorite compounds contain an excess of alkali and tend to raise the pH of the water. Calcium hypochlorite tablets are the predominant form in use in the United States for swimming pools. Sodium hypochlorite is the only liquid hypochlorite disinfectant in current use. There are several grades and proprietary forms available. Pound-for-pound of available chlorine, hypochlorite compounds have oxidizing powers equal to gas chlorine and can be employed for the same purposes in water treatment. Gas chlorination requires a larger initial investment for feed equipment than what is needed for hypochlorite compounds.

Calcium hypochlorite materials used in the water industry are chemically different from those materials variously marketed for many years as bleaching powder, chloride of lime, or chlorinated lime. Materials now in common use are high-test calcium hypochlorites containing about 70 percent available chlorine and marketed under several trade names.

High-test calcium hypochlorites are white corrosive solids that give off a strong chlorine odor. Granular powdered or tablet forms are commercially available and all are readily soluble in water.

Sodium hypochlorite is sold only as a liquid and is normally referred to as liquid bleach. It is generally available in concentrations of 5 to 15 percent available chlorine. These solutions are clear, light yellow, strongly alkaline, and corrosive in addition to having a strong chlorine smell.

High-test hypochlorites, though highly active, are relatively stable throughout production, packaging, distribution, and storage. Storage at 86° F. for a year may reduce the available chlorine by about 10 percent. Storing at lower temperatures reduces the loss. All sodium-hypochlorite solutions are unstable to some degree and deteriorate more rapidly than the dry compounds. Most producers recommend a shelf life of 60 to 90 days. Because light and heat accelerate decomposition, containers should be stored in a dry, cool, and dark area.

Disinfection Byproducts
Disinfection byproducts are formed when disinfectants used in water treatment plants react with bromide and/or natural organic matter (i.e., decaying vegetation) present in the source water. Different disinfectants produce different types or amounts of disinfection byproducts. Disinfection byproducts for which regulations have been established have been identified in drinking water, including trihalomethanes, haloacetic acids, bromate, and chlorite.

Trihalomethanes (THM)
Trihalomethanes (THM) are a group of four chemicals that are formed along with other disinfection byproducts when chlorine or other disinfectants used to control microbial contaminants in drinking water react with naturally occurring organic and inorganic matter in water. The trihalomethanes are chloroform, bromodichloromethane, dibromochloromethane, and bromoform. EPA has published the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate total trihalomethanes (TTHM) at a maximum allowable annual average level of 80 parts per billion. This standard will replace the current standard of a maximum allowable annual average level of 100 parts per billion in December 2001 for large surface water public water systems. The standard will become effective for the first time in December 2003 for small surface water and all ground water systems.

Haloacetic Acids (HAA5)
Haloacetic Acids (HAA5) are a group of chemicals that are formed along with other disinfection byproducts when chlorine or other disinfectants used to control microbial contaminants in drinking water react with naturally occurring organic and inorganic matter in water. The regulated haloacetic acids, known as HAA5, are: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid. EPA has published the Stage 1

Disinfectants/Disinfection Byproducts Rule to regulate HAA5 at 60 parts per billion annual average. This standard will become effective for large surface water public water systems in December 2001 and for small surface water and all ground water public water systems in December 2003.

Bromate is a chemical that is formed when ozone used to disinfect drinking water reacts with naturally occurring bromide found in source water. EPA has established the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate bromate at annual average of 10 parts per billion in drinking water. This standard will become effective for large public water systems by December 2001 and for small surface water and all ground public water systems in December 2003.

Chlorite
Chlorite is a byproduct formed when chlorine dioxide is used to disinfect water. EPA has published the Stage 1 Disinfectants/Disinfection Byproducts Rule to regulate chlorite at a monthly average level of 1 part per million in drinking water. This standard will become effective for large surface water public water systems in December 2001 and for small surface water and all ground water public water systems in December 2003.

Chloroform
Chloroform, typically the most prevalent THM measured in chlorinated water, is probably the most thoroughly studied disinfection byproduct. Toxicological studies have shown that high levels of chloroform can cause cancer in laboratory animals. Extensive research conducted since the early 1990s provides a clearer picture of what this means for humans exposed to far lower levels through drinking water.

One study (Larson et al. 1994a) conducted by the Centers for Health Research (CIIT) observed that a very large dose of chloroform, when given to mice once per day into the stomach (a procedure known as gavage), produced liver damage and eventually cancer. In a second CIIT cancer study (Larson et al., 1994b), mice were given the same daily dose of chloroform through the animals' drinking water. This time, no cancer was produced. Follow-up research showed that the daily gavage doses overwhelmed the capability of the liver to detoxify the chloroform, causing liver damage, cell death and regenerative cell growth, thereby increasing risks for cell mutation and cancer in exposed organs. When chloroform was given through drinking water, however, the liver could continually detoxify the chloroform as the mice sipped the water throughout the day. Without the initial liver toxicity, there was no cancer in the liver, kidney or other exposed organs (Butterworth et al., 1998).

In its most recent risk assessment, EPA considered the wealth of available information on chloroform, including the important work done at CIIT. EPA concludes that exposure to chloroform below the threshold level that causes cell damage is unlikely to increase the risk of cancer. While chloroform is likely to be carcinogenic at a high enough dose, exposures below a certain dose range are unlikely to pose any cancer risk to humans (US EPA, 2002a). For drinking water meeting EPA standards, chloroform is unlikely to be a health concern.

Sodium Chlorate
Sodium chlorate is a chemical compound with the chemical formula ($NaClO_3$). When pure, it is a white crystalline powder that is readily soluble in water. It is hygroscopic. It decomposes above 250 °C to release oxygen and leave sodium chloride. Industrially, sodium chlorate is synthesized from the electrolysis of a hot sodium chloride solution in a mixed electrode tank:

$$NaCl + 3H_2O - NaClO_3 + 3H_2$$

It can also be synthesized by passing chlorine gas into a hot sodium hydroxide solution. It is then purified by crystallization.

Chemical Oxygen Generation
Chemical oxygen generators, such as those in commercial aircraft, provide emergency oxygen to passengers to protect them from drops in cabin pressure by catalytic decomposition of sodium chlorate. The catalyst is normally iron powder. Barium peroxide (BaO_2) is used to absorb the chlorine which is a minor product in the decomposition. Iron powder is mixed with sodium chlorate and ignited by a charge which is activated by pulling on the emergency mask. The reaction produces more oxygen than is required for combustion. Similarly, the Solidox welding system used pellets of sodium chlorate mixed with combustible fibers to generate oxygen.

Toxicity in Humans
Due to its oxidative nature, sodium chlorate can be very toxic if ingested. The oxidative effect on hemoglobin leads to methaemoglobin formation, which is followed by denaturation of the globin protein and a cross-linking of erythrocyte membrane proteins with resultant damage to the membrane enzymes.

This leads to increased permeability of the membrane, and severe hemolysis. The denaturation of hemoglobin overwhelms the capacity of the G6PD metabolic pathway. In addition, this enzyme is directly denatured by chlorate reducing its activity. Therapy with ascorbic acid and methylene blue are frequently used in the treatment of methemoglobinemia. However, since methylene blue requires the presence of NADPH that requires normal functioning of G6PD system, it is less effective than in other conditions characterized by hemoglobin oxidation.

Acute severe hemolysis results, with multi-organ failure, including DIC and renal failure. In addition there is a direct toxicity to the proximal renal tubule. The treatment will consist of exchange transfusion, peritoneal dialysis or hemodialysis.

Developmental and Reproductive Effects
Several epidemiology studies have reported a possible association between disinfection byproducts and adverse reproductive outcomes, including spontaneous abortion (miscarriage). One study of women in several California communities (Waller et al. 1998) found a stronger association with bromodichloromethane (BDCM) than with other byproducts. Because the available studies have significant limitations, EPA and the American Water Works Association Research Foundation are sponsoring a new epidemiology study to replicate the 1998 Waller study.

When the Waller study was published, the available toxicology data on reproductive and developmental effects of some DBPs was quite limited. It was recognized that BDCM, in particular, should be thoroughly studied for a potential causal relationship to reproductive and developmental toxicity. The Research Foundation for Health and Environmental Effects , a tax-exempt foundation established by the Chlorine Chemistry Division of the American Chemistry Council, sponsored a set of animal studies (Christian et al. 2001, 2002) including two developmental toxicity studies on BDCM, a reproductive toxicity study on BDCM, and a reproductive toxicity study on dibromoacetic acid (DBA). The studies, published in the International Journal of Toxicology, found no adverse effects from BDCM and DBA at dose levels thousands of times higher than what humans are exposed to through drinking water. The studies were designed to comply with stringent EPA guidelines, and each study was independently monitored and peer reviewed.

Formulations
Sodium chlorate comes in dust, spray and granule formulations. There is a risk of fire and explosion in dry mixtures with other substances, especially organic materials, and other herbicides, sulfur, phosphorus, powdered metals, strong acids. In particular, when mixed with sugar, it has explosive properties. If accidentally mixed with one of these substances it should not be stored in human dwellings. Marketed formulations contain a fire retardant, but this has little effect if deliberately ignited. Most commercially available chlorate weedkillers contain

approximately 53% sodium chlorate with the balance being a fire depressant such as sodium metaborate or ammonium phosphates.

Sodium Chlorite
Sodium chlorite, like many oxidizing agents, should be protected from inadvertent contamination by organic materials to avoid the formation of an explosive mixture. The chemical explodes on percussive impact, and will ignite if combined with a strong reducing agent.

Toxicity
Sodium chlorite is a strong oxidant and can therefore be expected to cause clinical symptoms similar to the well-known sodium chlorate: methemoglobinemia, hemolysis, renal failure.[14] A dose of 10-15 grams of sodium chlorate can be lethal. Methemoglobemia had been demonstrated in rats and cats, and recent studies by the EMEA have confirmed that the clinical symptomatology is very similar to the one caused by sodium chlorate in the rat, mouse, rabbit, and the green monkey. There is only one human case in the medical literature of chlorite poisoning. It seems to confirm that the toxicity is equal to sodium chlorate. From the analogy with sodium chlorate, even small amounts of about 1 gram can be expected to cause nausea, vomiting and even life-threatening hemolysis in Glucose-6-Phosphate Dehydrogenase deficient persons. The EPA has set a maximum contaminant level of 1 milligram of chlorite per liter (1 mg/L) in drinking water.

Manufacture
The free acid, chlorous acid, $HClO_2$, is only stable at low concentrations. Since it cannot be concentrated, it is not a commercial product. However, the corresponding sodium salt, sodium chlorite, $NaClO_2$ is stable and inexpensive enough to be commercially available. The corresponding salts of heavy metals (Ag+, Hg+, Tl+, Pb2+, and also Cu2+ and NH_4+) decompose explosively with heat or shock. Sodium chlorite is derived indirectly from sodium chlorate, $NaClO_3$. First, the explosive (only at concentrations greater than 10% in atmosphere) chlorine dioxide, ClO_2 is produced by reducing sodium chlorate in a strong acid solution with a suitable reducing agent (for example, sodium sulfite, sulfur dioxide, or hydrochloric acid). The chlorine dioxide is then absorbed into an alkaline solution and reduced with hydrogen peroxide (H_2O_2), yielding sodium chlorite.

Stachybotrys
Stachybotrys is a genus of molds, or asexually-reproducing, filamentous fungi. Closely related to the genus Memnoniella, most Stachybotrys species inhabit materials rich in cellulose. The genus has a widespread distribution, and contains about 50 species. The most infamous species, S. chartarum (also known as S. atra) and S. chlorohalonata are known as "black mold" or "toxic black mold" in the U.S. and are frequently associated with poor indoor air quality that arises after fungal growth on water-damaged building materials

Symptoms of Stachybotrys Exposure in Humans
Exposure to the mycotoxins present in Stachybotrys chartarum or Stachybotrys atra can have a wide range of effects. Depending on the length of exposure and volume of spores inhaled or ingested, symptoms can manifest as chronic fatigue or headaches, fever, irritation to the eyes, mucous membranes of the mouth, nose and throat, sneezing, rashes, and chronic coughing. In severe cases of exposure or cases exacerbated by allergic reaction, symptoms can be extreme including nausea, vomiting, and bleeding in the lungs and nose.

Understanding Commonly Used Water Disinfectants
Almost all U.S. systems that disinfect their water use some type of chlorine-based process, either alone or in combination with other disinfectants. In addition to controlling disease-causing organisms, chlorination offers a number of benefits including:

- Reduces many disagreeable tastes and odors;
- Eliminates slime bacteria, molds and algae that commonly grow in water supply reservoirs, on the walls of water mains and in storage tanks;

- Removes chemical compounds that have unpleasant tastes and hinder disinfection; and
- Helps remove iron and manganese from raw water.

As importantly, only chlorine-based chemicals provide "residual disinfectant" levels that prevent microbial re-growth and help protect treated water throughout the distribution system.

The Risks of Waterborne Disease
Where adequate water treatment is not readily available, the impact on public health can be devastating. Worldwide, about 1.2 billion people lack access to safe drinking water, and twice that many lack adequate sanitation. As a result, the World Health Organization estimates that 3.4 million people, mostly children, die every year from water-related diseases.

Even where water treatment is widely practiced, constant vigilance is required to guard against waterborne disease outbreaks. Well-known pathogens such as E. coli are easily controlled with chlorination, but can cause deadly outbreaks given conditions of inadequate or no disinfection. A striking example occurred in May 2000 in the Canadian town of Walkerton, Ontario. Seven people died and more than 2,300 became ill after E. coli and other bacteria infected the town's water supply. A report published by the Ontario Ministry of the Attorney General concludes that, even after the well was contaminated, the Walkerton disaster could have been prevented if the required chlorine residuals had been maintained.

Some emerging pathogens such as Cryptosporidium are resistant to chlorination and can appear even in high quality water supplies. Cryptosporidium was the cause of the largest reported drinking water outbreak in U.S. history, affecting over 400,000 people in Milwaukee in April 1993. More than 100 deaths are attributed to this outbreak. New regulations from the U.S. Environmental Protection Agency (EPA) will require water systems to monitor Cryptosporidium and adopt a range of treatment options based on source water Cryptosporidium concentrations. Most water systems are expected to meet EPA requirements while continuing to use chlorination.

The Benefits of Chlorine
Potent Germicide
Chlorine disinfectants can reduce the level of many disease-causing microorganisms in drinking water to almost immeasurable levels. Chlorine is added to drinking water to destroy pathogenic (disease-causing) organisms. It can be applied in several forms: elemental chlorine (chlorine gas), sodium hypochlorite solution (bleach) and dry calcium hypochlorite.

When applied to water, each of these forms "free chlorine" (see Sidebar: How Chlorine Kills Pathogens). One pound of elemental chlorine provides approximately as much free available chlorine as one gallon of sodium hypochlorite (12.5% solution) or approximately 1.5 pounds of calcium hypochlorite (65% strength). While any of these forms of chlorine can effectively disinfect drinking water, each has distinct advantages and limitations for particular applications. Almost all water systems that disinfect their water use some type of chlorine-based process, either alone or in combination with other disinfectants.

Taste and Odor Control
Chlorine disinfectants reduce many disagreeable tastes and odors. Chlorine oxidizes many naturally occurring substances such as foul-smelling algae secretions, sulfides and odors from decaying vegetation.

Biological Growth Control
Chlorine disinfectants eliminate slime bacteria, molds and algae that commonly grow in water supply reservoirs, on the walls of water mains and in storage tanks.

Chemical Control
Chlorine disinfectants destroy hydrogen sulfide (which has a rotten egg odor) and remove ammonia and other nitrogenous compounds that have unpleasant tastes and hinder disinfection. They also help to remove iron and manganese from raw water.

Water Treatment
Every day, approximately 170,000 (U.S. EPA, 2002) public water systems treat and convey billions of gallons of water through approximately 880,000 miles (Kirmeyer, 1994) of distribution system piping to U.S. homes, farms and businesses. Broadly speaking, water is treated to render it suitable for human use and consumption. While the primary goal is to produce a biologically (disinfected) and chemically safe product, other objectives also must be met, including: no objectionable taste or odor; low levels of color and turbidity (cloudiness); and chemical stability (non-corrosive and non-scaling). Individual facilities customize treatment to address the particular natural and manmade contamination characteristic of their raw water.

Surface water usually presents a greater treatment challenge than groundwater, which is naturally filtered as it percolates through sediments. Surface water is laden with organic and mineral particulate matter, and may harbor protozoan parasites such as Cryptosporidium parvum and Giardia lamblia.

Water Distribution
In storage and distribution, drinking water must be kept safe from microbial contamination. Frequently, slippery films of bacteria, known as biofilms, develop on the inside walls of pipes and storage containers. Among disinfection techniques, chlorination is unique in that a pre-determined chlorine concentration may be designed to remain in treated water as a measure of protection against harmful microbes encountered after leaving the treatment facility. In the event of a significant intrusion of pathogens resulting, for example, from a broken water main, the level of the average “chlorine residual” will be insufficient to disinfect contaminated water. In such cases, it is the monitoring of the sudden drop in the chlorine residual that provides the critical indication to water system operators that there is a source of contamination in the system.

The Challenge of Disinfection Byproducts
While protecting against microbial contamination is the top priority, water systems must also control disinfection byproducts (DBPs), chemical compounds formed unintentionally when chlorine and other disinfectants react with natural organic matter in water. In the early 1970s, EPA scientists first determined that drinking water chlorination could form a group of byproducts known as trihalomethanes (THMs), including chloroform. EPA set the first regulatory limits for THMs in 1979. While the available evidence does not prove that DBPs in drinking water cause adverse health effects in humans, high levels of these chemicals are certainly undesirable. Cost-effective methods to reduce DBP formation are available and should be adopted where possible.

Chemical Safety (IPCS 2000) Strongly Cautions:
The health risks from these byproducts at the levels at which they occur in drinking water are extremely small in comparison with the risks associated with inadequate disinfection. Thus, it is important that disinfection not be compromised in attempting to control such byproducts. Recent EPA regulations have further limited THMs and other DBPs in drinking water. Most water systems are meeting these new standards by controlling the amount of natural organic material prior to disinfection.

Chlorine and Water System Security
The prospect of a terrorist attack has forced all water systems, large and small, to re-evaluate and upgrade existing security measures. Since September 11th, 2001, water system managers have taken unprecedented steps to protect against possible attacks such as chemical or biological contamination of the water supply, disruption of water treatment or distribution, and intentional release of treatment chemicals.

With passage of the Public Health Security and Bioterrorism Response Act of 2002, Congress required community water systems to assess their vulnerability to a terrorist attack and other intentional acts. As part of these vulnerability assessments, systems assess the transportation, storage and use of treatment chemicals. These chemicals are both critical assets (necessary for delivering safe water) and potential vulnerabilities (may pose significant hazards, if released). Water systems using elemental chlorine, in particular, must determine whether existing protection systems are adequate. If not, they must consider additional measures to reduce the likelihood of an attack or to mitigate the potential consequences.

Disinfection is crucial to water system security, providing the “front line” of defense against biological contamination. However, conventional treatment barriers in no way guarantee safety from biological attacks. Additional research and funding are needed to improve prevention, detection and responses to potential threats.

The Future of Chlorine Disinfection

Despite a range of new challenges, drinking water chlorination will remain a cornerstone of waterborne disease prevention. Chlorine’s wide array of benefits cannot be provided by any other single disinfectant. While alternative disinfectants (including chlorine dioxide, ozone, and ultraviolet radiation) are available, all disinfection methods have unique benefits, limitations, and costs. Water system managers must consider these factors, and design a disinfection approach to match each system’s characteristics and source water quality.

Understanding Giardia lamblia

Giardia lamblia, discovered approximately 20 years ago, is another emerging waterborne pathogen. This parasitic microorganism can be transmitted to humans through drinking water that might otherwise be considered pristine. In the past, remote water sources that were not affected by human activity were thought to be pure, warranting minimal treatment. However, it is known now that all warm-blooded animals may carry Giardia and that beaver are prime vectors for its transmission to water supplies.

There is a distinct pattern to the emergence of new pathogens. First, there is a general recognition of the effects of the pathogen in highly susceptible populations such as children, cancer patients and the immunocompromised. Next, practitioners begin to recognize the disease and its causative agent in their own patients, with varied accuracy. At this point, some may doubt the proposed agent is the causative agent, or insist that the disease is restricted to certain types of patients. Finally, a single or series of large outbreaks result in improved attention to preventive efforts. From the 1960's to the 1980's this sequence of events culminated in the recognition of Giardia lamblia as a cause of gastroenteritis (Lindquist, 1999).

Understanding Waterborne Diseases
Detection and investigation of waterborne disease outbreaks is the primary responsibility of local, state and territorial public health departments, with voluntary reporting to the CDC. The CDC and the U.S. Environmental Protection Agency (EPA) collaborate to track waterborne disease outbreaks of both microbial and chemical origins. Data on drinking water and recreational water outbreaks and contamination events have been collected and summarized since 1971.

While useful, statistics derived from surveillance systems do not reflect the true incidence of waterborne disease outbreaks because many people who fall ill from such diseases do not consult medical professionals. For those who do seek medical attention, attending physicians and laboratory and hospital personnel are required to report diagnosed cases of waterborne illness to state health departments. Further reporting of these illness cases by state health departments to the CDC is voluntary, and statistically more likely to occur for large outbreaks than small ones.

Despite these limitations, surveillance data may be used to evaluate the relative degrees of risk associated with different types of source water and systems, problems in current technologies and operating conditions, and the adequacy of current regulations. (Craun, Nwachuku, Calderon, and Craun, 2002).

Understanding Disinfection Byproducts (DBPS)

Chlorine and other chemical disinfectants have been widely used by public water systems (along with filtration) to protect the public from microbial pathogens in drinking water. DBPs are formed when certain disinfectants react with DBP precursors (organic and inorganic materials) in source waters. In most cases, natural organic matter (NOM) is an important factor that affects the levels of DBPs that form (NOM is usually measured as TOC). The levels of DBPs in drinking water can vary significantly from one point in a distribution system to another, as many continue to form in the distribution system. DBP levels are generally higher in surface water systems because surface water usually contains higher DBP precursor levels and requires stronger disinfection.

Updating the Safe Drinking Water Act Regulations
EPA has regulated DBPs in drinking water since 1979. The first DBP standards limited THM levels to 100 parts per billion (ppb) for systems serving more the 10,000 people. In the 1996 Safe Drinking Water Act (SDWA) reauthorization, Congress called for EPA to revise its standards for disinfectants and DBPs in two stages. The revised regulations are designed to reduce potential DBP risks, while ensuring that drinking water is protected from microbial contamination.

Stage 1 DBP Rule
In December 1998 USEPA issued the Stage 1 Disinfectants and Disinfection Byproducts (Stage 1 DBP) rule. The regulations are based on an agreement between members of a Federal Advisory Committee that included representatives from water utilities, the Chlorine Chemistry Division of the American Chemistry Council, public health officials, environmentalists and other stakeholder groups. This diverse group of experts developed a consensus set of recommendations to cost-effectively reduce DBP levels, without compromising protection from microbial contaminants.

The Stage 1 DBP rule mandates a process called enhanced coagulation to remove natural organic matter, reducing the potential for DBPs to form. The rule also sets enforceable Maximum Contaminant Levels (MCLs) for total trihalomethanes at 80 ppb and the sum of five Haloacetic Acids (HAAs) at 60 ppb. These MCLs are based on system-wide running annual averages, meaning that concentrations may be higher at certain times and at certain points in the system, as long as the system-wide average for the year is below the MCL. In developing the Stage 1 DBP rule, EPA was very cautious about encouraging the use of alternative disinfectants. The Agency recognized that alternative disinfectants might reduce THMs and HAAs, but produce other, less understood, byproducts. The Agency also avoided making recommendations that would encourage utilities to reduce the level of disinfection currently being practiced.

Large water systems (those serving more than 10,000 persons) were required to comply with the Stage 1 DBP rule by December 2001. Systems serving fewer than 10,000 persons must comply by December 2003.

Stage 2 DBP Rule
As the Stage 1 rule is coming into full force, EPA is completing work on its Stage 2 DBP rule. The Stage 2 rule is being developed simultaneously with the Long Term 2 Enhanced Surface Water Treatment Rule (LT2) in order to address the risk trade-offs between pathogen control and exposure to DBPs. The LT2 rule deals primarily with controlling Cryptosporidium and other resistant pathogens discussed in Chapter 3. Again, the EPA sought recommendations from an advisory group, the Stage 2 Microbial and Disinfection Byproducts Federal Advisory Committee.

As outlined in the advisory committee's September 2000 Agreement in Principle, the MCLs for THMs and five HAAs will remain 80 ppb and 60 ppb respectively, based on each utility's system-wide running annual averages. However, the Stage 2 rule will also limit DPB levels at specific locations within distribution systems. When fully implemented, these locational running annual average limits will mean that no part of the distribution system will be allowed to exceed the MCLs for these substances.

Total Trihalomethanes
Trihalomethanes (THMs) are chemical compounds in which three of the four hydrogen atoms of methane (CH4) are replaced by halogen atoms. Many trihalomethanes find uses in industry as solvents or refrigerants. THMs are also environmental pollutants, and many are considered carcinogenic. Trihalomethanes with all the same halogen atoms are called haloforms. Trihalomethanes are formed as a by-product predominantly when chlorine is used to disinfect water for drinking. They represent one group of chemicals generally referred to as disinfection by-products. They result from the reaction of chlorine and/or bromine with organic matter present in the water being treated. The THMs produced have been associated through epidemiological studies with some adverse health effects. Many governments set limits on the amount permissible in drinking water. However, trihalomethanes are only one group of many hundreds of possible disinfection by-products—the vast majority of which are not monitored—and it has not yet been clearly demonstrated which of these are the most plausible candidate for causation of these health effects. In the United States, the EPA limits the total concentration of the four chief constituents (chloroform, bromoform, bromodichloromethane, and dibromochloromethane), referred to as total trihalomethanes (TTHM), to 80 parts per billion in treated water.

THM Treatment
THM levels tend to increase with pH, temperature, time, and the level of "precursors" present. Precursors are organic material which reacts with chlorine to form THM's. One way to decrease THM's is to eliminate or reduce chlorination before the filters and to reduce precursors. There are more precursors present before filtration, so we want to reduce or eliminate the time chlorine is in contact with this water. If some oxidation before filtration is required, an alternative disinfectant like potassium permanganate or peroxide could be considered. Note that this may not be an option if prechlorination is necessary to achieve required CT values.

The EPA has indicated that the best available technology for THM control at treatment plants is removal of precursors through "enhanced coagulation". Enhanced coagulation refers to the process of optimizing the filtration process to maximize removal of precursors. Removal is improved by decreasing pH (to levels as low as 4 or 5), increasing the feed rate of coagulants, and possibly using ferric coagulants instead of alum.

Understanding Cryptosporidiosis
Cryptosporidium is an emerging parasitic protozoan pathogen because its transmission has increased dramatically over the past two decades. Evidence suggests it is newly spread in increasingly popular day-care centers and possibly in widely distributed water supplies, public pools and institutions such as hospitals and extended-care facilities for the elderly. Recognized in humans largely since 1982 and the start of the AIDS epidemic, Cryptosporidium is able to cause potentially life-threatening disease in the growing number of immunocompromised patients. Cryptosporidium was the cause of the largest reported drinking water outbreak in U.S. history, affecting over 400,000 people in Milwaukee in April, 1993. More than 100 deaths are attributed to this outbreak. Cryptosporidium remains a major threat to the U.S. water supply (Ibid.).

The EPA is developing new drinking water regulations to reduce Cryptosporidium and other resistant parasitic pathogens. Key provisions of the Long Term 2 Enhanced Surface Water Treatment Rule include source water monitoring for Cryptosporidium; inactivation by all unfiltered systems; and additional treatment for filtered systems based on source water Cryptosporidium concentrations. EPA will provide a range of treatment options to achieve the inactivation requirements. Systems with high concentrations of Cryptosporidium in their source water may adopt alternative disinfection methods (e.g., ozone, UV, or chlorine dioxide). However, most water systems are expected to meet EPA requirements while continuing to use chlorination. Regardless of the primary disinfection method used, water systems must continue to maintain residual levels of chlorine-based disinfectants in their distribution systems.

Understanding Bacteriological Monitoring

26 waterborne-disease outbreaks have been documented each year in the United States over the past 25 years (Kramer and others, 1996). The persistence of outbreaks over time indicates that more progress is needed to meet the “drinkable and swimmable” goals of Federal water-quality legislation. Although significant improvements in drinking water and wastewater treatment have been achieved, waterborne disease outbreaks indicate that certain types and sources of waterborne pathogens (disease-causing organisms) are still a threat to human health in the United States (Craun, 1992). In particular, waterborne disease outbreaks caused by Escherichia coli O157.:H7 were reported more frequently in 1995-96 than in previous years, and during that same period, Cryptosporidium and Giardia caused large outbreaks associated with recreational water quality (Levy and others, 1998).

Microbiological examination of water is used to determine the sanitary quality of water and the public health risk from waterborne disease. Although microbiological monitoring of finished waters is well established, microbiological monitoring of source waters and recreational waters is considered by some to be fragmented, incomplete, or virtually nonexistent in many parts of the Nation (Rose and others, 1999). Data to characterize the microbiological quality of source waters are usually collected for local purposes, most often to judge compliance with standards for protection of public health in swimmable or drinkable waters. For example, monitoring programs vary widely at the local level for recreational waters, and the result is the inconsistent use of indicator organisms across the United States (U.S. Environmental Protection Agency, 1999a).

There is a need to identify human and animal factors associated with contamination of different source and recreational waters and to understand the processes that affect microbiological water quality. Concepts about the relation between the occurrence and distribution of microbiological contaminants and a range of environmental factors such as climate, hydrology, land use, and human and animal population densities need to be tested in areas that represent the national water-use patterns for public and domestic supply and for recreational uses.

Understanding Bacteriological Monitoring
Understanding Bacteria Sampling

Waterborne bacterial pathogens in the United States include species in the genera Salmonella, Shigella, Vibrio, Campylobacter, Yersinia, and pathogenic strains of E. coli. Because bacterial pathogens generally appear intermittently in low concentrations in the environment and because methods of culturing are difficult, fecal-indicator bacteria are used to indicate the possible presence of pathogens. The most widely used bacterial indicators include total coliforms, fecal coliforms, E. coli, fecal streptococci, enterococci, and Clostridium perfringens (C. perfringens). A good indicator organism should be applicable in all types of water; unable to reproduce in ambient waters; be harmless to man and other animals; lend itself to easy, quantitative testing procedures; be of warm-blooded animal origin; correlate with fecal contamination; and be present in waters in greater numbers than and survive as long as or longer than pathogens.

The historical definition of the total-coliform group has been based on the method used for detection (lactose fermentation) rather than on systematic bacteriology (American Public Health Association and others, 1998). Total coliforms are defined as aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation at $35^{O}C$ within 48 hours (Britton and Greeson,1989). Elevated temperature tests identify those genera of total coliform bacteria that belong in the more specific fecal-coliform group. Fecal coliforms are total coliforms capable of producing gas from lactose at $44.5^{O}C$.

Escherichia coli is a species of the fecal-coliform group. Total coliforms include several genera that are found in the human intestine; however, some genera are also found in soils, on vegetation, and in industrial wastes. This multiplicity of sources makes the sanitary significance of total coliforms difficult to establish (Palmer and others, 1984).

They are used as a rough measure of source-water quality and as a screen for fecal contamination. In addition, speciation of the total-coliform group may provide information on treatment effectiveness and the source of colonization of a distribution system or well (American Public Health Association, 1998, p. 9-1). The fecal-coliform indicator used to assess fecal contamination of water has been faulted because of nonfecal sources of at least one member of the fecal coliform group. For example, thermotolerant Klebsiella species have been observed in pulp- and papermill effluents, textile-processing-plant effluent, cotton-mill wastewaters, and sugar-beet wastes, in the absence of fecal contamination (U.S. Environmental Protection Agency, 1986a).

Alternatively, E. coli is a natural inhabitant of the gastrointestinal tract of warm-blooded animals and is direct evidence of fecal contamination from warm-blooded animals. The fecal streptococci are a group of fecal-indicator bacteria that include a variety of species and strains that are all gram positive cocci. Although the normal habitat of fecal streptococci is the gastrointestinal tract of warm-blooded animals, some species are not exclusive to animals (American Public Health Association, 1998, p. 9-74). In fact, studies on the distribution of fecal streptococci in water indicate that at least one strain commonly found in environmental samples is ubiquitous and can exist for extended periods in soil and water (Geldreich, 1976). Fecal streptococci, therefore, have limited value as an indicator of fecal contamination in environmental samples. The enterococci group is a subgroup of the fecal streptococci, and it is considered a more specific indicator of fecal contamination.

The enterococci are differentiated from other streptococci by their ability to grow in 6.5 percent chloride, at pH 9.6, and at elevated temperatures. The enterococci method is valuable for determining the extent of fecal contamination of recreational surface waters, especially marine waters (American Public Health Association, 1998, p. 9-75).

In addition, because enterococccci cells are a different shape and have different survival rates than members of the coliform group, enterococci may be useful in assessing transport of fecal contamination in ground water. Clostridium perfringens is present in large numbers in human and animals wastes, and its spores are more resistant to disinfection and environmental stresses than is E. coli. Clostridium perfringens has been suggested as a conservative tracer of past fecal contamination and as an indicator for chlorinated water in distribution systems (Bisson and Cabelli, 1980).

Clostridium perfringens, however, is probably not an appropriate indicator for most recreational waters because spores in the sediment are resuspended into the water column from swimmer or wave disturbances (Bisson and Cabelli, 1980). One exception is that C. perfringens may be a reliable indicator of streamwater quality in tropical climates, where warm water temperatures support the growth and reproduction of E. coli and aerobic conditions preclude the growth and sporulation of C. perfringens (Fujioka and Shizumura, 1985).

Clostridium perfringens has also been found to be a sensitive indicator of microorganisms entering streams from point sources but not a reliable indicator of nonpoint sources (Sorenson and others, 1989). Detection of C. perfringens in water has been proposed as an indicator of the presence and density of pathogenic viruses and possibly other stress resistant microorganisms (U.S. Environmental Protection Agency, 1996c).

Protozoan Pathogens

The principal protozoan pathogens that affect the public health acceptability of waters in the United States are Giardia lamblia (Giardia) and Cryptosporidium parvum (Cryptosporidium). These organisms are widely distributed in the aquatic environment and have been implicated in several recent outbreaks of waterborne disease, including a well-publicized outbreak of cryptosporidiosis in Milwaukee, Wisconsin (Rose and others, 1997). Both Giardia and Cryptosporidium produce environmentally resistant forms (called cysts and oocysts), which allow for the extended survival of the parasites in water and treated water.

Because cysts and oocysts are more resistant to disinfection and survive longer in the environment than bacterial indicators, fecal-indicator bacteria are not adequate indicators for Giardia and Cryptosporidium in source waters. The presence of protozoan pathogens in water, therefore, must be verified by identification of the pathogens themselves. The USEPA-required method for detection of Giardia and Cryptosporidium in source and drinking water under the ICR involves nominal porosity filtration and indirect fluorescent antibody procedures (U.S. Environmental Protection Agency, 1996c). The ICR method has been criticized for being difficult to implement, being characterized by poor recovery of target organisms, and yielding highly variable results (U.S. Environmental Protection Agency, 1996b). As a result, the USEPA supported the development of Method 1622 for Cryptosporidium (U.S. Environmental Protection Agency, 1998b), and Method 1623 for Giardia and Cryptosporidium (U.S. Environmental Protection Agency, 1999c). Method 1622 was validated through an interlaboratory study and revised as a final, valid method in January 1999.

Understanding Routine Coliform Sampling
Streamwater sample collection
When designing a sampling plan, consider that the spatial and temporal distribution of microorganisms in surface water can be as variable as the distribution of suspended sediment because microorganisms are commonly associated with solid particles. The standard samplers can be used to collect streamwater samples for bacterial and viral indicators, *Cryptosporidium*, and *Giardia* providing that the equipment coming in contact with the water is properly cleaned and sterilized. For streamwater samples, these include the US-D77TM, US-D95, US-DH81, and weighted- and open-bottle samplers with autoclavable Teflon, glass, or polypropylene components.

• Prepare a separate set of sterile equipment (bottles nozzles, and caps) for sampling at each site.

• Follow sampling techniques given in Shelton (1994) to ensure that a sample is representative of the flow in the cross section. Use equal-width increment (EWI) or equal-discharge-increment (EDI) methods described in Edwards and Glysson (1988), unless site characteristics dictate otherwise.

• Because churn and cone splitters cannot be autoclaved, use a sterile 3-L bottle to composite subsamples for bacterial and viral indicators when using EDI and EWI methods. If possible, composite by collecting subsamples at vertical locations in the cross section without overfilling the bottle.

• Alternatively, if the stream depth and (or) velocity is not sufficient to use depth-width integrating techniques, collect a sample by a hand-dip method (Myers and Sylvester, 1997).

• Collect approximately 1 L of streamwater for bacterial and viral indicators. Process the sample for *E. coli* and enterococci; send the remainder (at least 500 mL) on ice to the laboratory for *C. perfringens* and coliphage analysis.

Method 1623
For *Cryptosporidium* and *Giardia* analysis by Method 1623 (U.S. Environmental Protection Agency, 1999c), collect 10 L of streamwater for each protozoan pathogen using standard sampling techniques described in Myers and Sylvester (1997). Special sterilization procedures are needed for equipment used in the collection of samples for *Cryptosporidium* and *Giardia*.

Autoclaving is not effective in neutralizing the epitopes on the surfaces of the oocysts and cysts that will react with the antibodies used for detection.

• Wash and scrub the equipment with soap and warm tap water to remove larger particulates and rinse with deionized water. Submerge the equipment in a vessel containing 12 percent hypochlorite solution for 30 minutes. Wash the equipment free of residual sodium hypochlorite solution with three rinses of filter-sterilized water; do not de-chlorinate the equipment using sodium thiosulfate. This procedure is best done in the office with dedicated sampling equipment for each site; however, it may be done in the field as long as the hypochlorite solution is stored and disposed of properly.
• Composite the sample in a 10-L cubitainer that is pre-sterilized by the manufacturer. The cubitainer is sent in a cardboard box to laboratory for *Cryptosporidium* analysis. The sample does not have to be kept on ice during transport. At this time, two methods are recommended for analysis of water samples for enteric viruses: (1) the reverse-transcriptase, polymerase chain reaction (RTPCR) method (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997) and (2) the cell-culture method (U.S. Environmental Protection Agency, 1996c). Sampling and equipment cleaning procedures are more thoroughly described elsewhere (G. Shay Fout, U.S. Environmental Protection Agency, 1997; U.S. Environmental Protection Agency, 1996c). Briefly, 100 L of streamwater is pumped by means of a specially designed sampling apparatus and passed through a Virosorb1 1MDS filter (Cuno, Meriden, Conn.). The sampling equipment is obtained from the analyzing laboratory; for example, the USGS Ohio District Laboratory has modified the sampling apparatus (G. Shay Fout, U.S. Environmental Protection Agency, 1997) into a self-contained box with easy-to-use control valves. The 1MDS filters, which remove viruses present in the water by charge interactions, are kept on ice and sent to a central laboratory for virus elution, concentration, and detection.

Groundwater Sample Collection
Collecting ground-water samples by use of sterile techniques requires knowledge of the type of well, its use, its construction, and its condition.
• Swab the electronic tape used for water-level measurements with isopropyl or ethyl alcohol.
• In sampling subunit survey wells, once purging criteria have been met as described in Koterba and others (1995), collect the sample directly from the tap into a sterile container.
• Remove screens, filters, other devices from the tap before collecting the sample, and do not sample from leaking taps. Because we are interested in the microbial population in the ground water and not in the distribution system, it is best to sample directly from the wellhead using a pump with sterile tubing, if possible. Because this is operationally prohibitive for private domestic wells, a tap that yields water directly from the well and before entering the holding tank is preferred. Water collected after treatment is unsuitable for microbiological analysis.
• Document the stage of the distribution system from which water was collected and details about the distribution system, including the type of tank and condition of the tank and pipes. In addition, if the well can easily be opened for inspection, document the condition of the well, including the sanitary seal (if any) and the amount of debris in the well. Any information on the location of the well, including proximity to septic systems or feedlots, should also be documented in the field at the time of sampling.

For wells without in-place pumps, samples should be obtained by use of the following methods
(in descending order from most to least desirable):
(1) a peristaltic or vacuum pump with autoclavable silicon tubing, (2) a sterile bailer, (3) a chlorine-disinfected pump and tubing, or (4) a detergent-cleaned pump and tubing. Pre-sampling activities, such as purging, must be carried out in such a way as to avoid contaminating the well. All equipment must be properly cleaned and sterilized between sites, using a Liquinox wash and a thorough tap water or deionized-water rinse. If using this last method, collect additional field blanks to evaluate the effectiveness of the cleaning procedure. Refer to Myers and Sylvester (1997) for a detailed discussion of ground-water sampling for microbiological analysis.

Because ground water is less prone to microbiological contamination than surface water, larger volumes of ground water are needed than of surface water.

• For regular sampling, collect 3 L of ground water for bacterial and viral indicators.

• Process the sample for total coliforms, *E. coli*, and enterococci using 200-mL sample volumes for each analysis; send the remainder (at least 2.5 L) to the laboratory for coliphage analysis. In the laboratory, coliphage analysis is done using 1 L for somatic and 1 L for F-specific coliphage.

• For enteric virus analysis by RT-PCR and cell culture, use the same sampler for ground-water samples as for streamwater samples; pump 2,000 L of ground water through the sampling apparatus and 1MDS filter.

Sample Preservation and Storage

Holding times for samples before processing are 6 hours for total coliforms, *E. coli*, and enterococci and 24 hours for *C. perfringens*, coliphage, *Cryptosporidium*, *Giardia*, and the 1MDS filters for enteric viruses by RTPCR and cell culture.

• After collection, immediately store the sample on ice.

• Be sure to keep the sample out of direct sunlight, because ultraviolet rays kill microorganisms.

• Add sodium thiosulfate to sample bottles for bacterial and viral indicators if the water collected contains residual chlorine. (Samples may have residual chlorine if the sampling site is downstream from a wastewater-treatment plant that chlorinates its effluents). Add ethylene diaminetetracetic acid to sample bottles when water is suspected to contain trace elements such as copper, nickel, and zinc at concentrations greater than 1 mg/L (Britton and Greeson, 1989, p. 5-6; U.S. Environmental Protection Agency, 1978, p. 6; American Public Health Association and others, 1998, p. 9-19). (Sodium thiosulfate or ethylene diaminetetracetic acid are not added to containers for *Cryptosporidium* and *Giardia)*.

Analytical Methods

Field Analysis

Analysis of water samples for total coliforms, *E. coli,* and enterococci, are done by use of membrane filtration (MF) or most-probable number (MPN) methods. Because membrane filtration is easier to use and provides a more precise quantification of bacteria than MPN, MF is recommended for most analyses. Refer to Myers and Sylvester (1997) for complete MF procedures.

Different MF methods are used for quantification of bacteria in ground-water and streamwater samples.

• For examining streamwater samples for *E. coli*, use the USEPA-recommended mTEC agar method (Environmental Protection Agency, 1986b).

• For examining ground-water samples for total coliforms and *E. coli*, use the MI method (Brenner and others, 1993).

• For enterococci, use the mEI method (U.S. Environmental Protection Agency, 1997).

• For streamwater, plate sufficient sample volumes in order to obtain at least one plate in the ideal count range. For ground water, a 200-mL sample volume is usually sufficient.

Testing of new microbiological monitoring methods and comparing the recoveries of new methods to the USEPA-approved method can be done by use of the NAWQA network. For ground-water samples, for example, one may include a commercially available MPN kit, Colilert (Idexx Laboratories, Westbrook, Maine), for simultaneous detection of total coliforms and *Escherichia coli*. For streamwater sampling, one may include a single-step modified Mtec medium with 5-bromo-6-chloro-3-indolyl'β-d-glucuronide (Bennett Smith, USEPA, Cincinnati, Ohio, oral commun., 1997); this method was developed to replace the mTEC method. Other new methods can be added to the monitoring program for field testing as they are developed.

Laboratory Analysis

Samples need to be kept on ice and shipped to a central laboratory for analysis of coliphage, *C. perfringens*, *Cryptosporidium, Giardia*, and enteric viruses by the current analytical methods. The single-agar layer (SAL), direct plating method with induction of β-galactosidase (Ijzerman and Hagedorn, 1992) is recommended for detection of somatic and F-specific coliphage in streamwater samples. In this method, 100-mL sample volumes are mixed with an agar medium, *E. coli* host culture, chemicals that induce the β-galactosidase enzyme, and appropriate antibiotics. The mixtures are poured into four 150- x 15-mm plates and incubated at 35°C.

Upon infection by coliphage in the water sample, the *E. coli* host cells are lysed and stable indolyl product that is dark blue is visible within each plaque. Viral plaques are easily identified and enumerated by the distinct blue circle. Because of contamination by naturally occurring bacteria in streamwater samples, antibiotic- resistant host-culture strains, *E. coli* CN-13 (resistant to nalidixic acid) and *E. coli* F-amp (resistant to streptomycin and ampicillin) are used as hosts for somatic and F-specific coliphage, respectively. Large sample volumes, such as 1-L volumes or greater, are recommended for detection of coliphage in ground water. Because the SAL method is impractical for sample volumes above 100 mL, an alternative method should be used for ground-water sample analysis.

One example, currently being tested by USEPA, is a two-step enrichment presence-absence method (U.S. Environmental Protection Agency, 1999e). Samples for enumeration of *C. perfringens* are analyzed by use of the mCP agar method (U.S. Environmental Protection Agency, 1996c). Standard MF techniques are used, and the plates are incubated anaerobically for 24 hours at 44.5°C. After incubation, the plates are exposed to ammonium hydroxide, and all straw-colored colonies that turn dark pink to magenta are counted as *C. perfringens*. In the laboratory, *C. perfringens* analyses are done on 100-, 30-,and 10-mL volumes of streamwater. In the case of a high-flow or high-turbidity streamwater sample, lower sample volumes may be plated.

Method 1623 (U.S. Environmental Protection Agency, 1999c) is recommended for detection of *Cryptosporidium* oocysts and *Giardia* cysts in water. The oocysts are concentrated on a capsule filter from a 10-L water sample, eluted from the capsule filter with buffer, and concentrated by centrifugation. Immunomagnetic separation (IMS) is used to separate the oocysts from other particulates in the sample. In IMS, the oocysts are magnetized by attachment of magnetic beads conjugated to an antibody and then are separated from sediment and debris by means of a magnet.

Fluorescently labeled antibodies and vital dye are used to make the final microscopic identification of oocysts and cysts. The reverse-transcriptase, polymerase chain reaction (RT-PCR) and cell-culture methods are recommended for detection of enteric viruses in water samples (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c). To prepare samples for RT-PCR and cell culture, attached viruses are eluted from a 1MDS filter with beef extract (pH 9.5), concentrated using celite (pH 4.0), and eluted with sodium phosphate (pH 9.5).

For RT-PCR analysis, viruses are isolated from the eluate by ultracentrifugation through a sucrose gradient, and trace contaminants are removed by extraction with a solvent mixture. During these steps, the 10-L streamwater sample (or 2,000-L ground-water sample) is concentrated down to 40 µL. An aliquot of the concentrate is used for RT-PCR, wherein any target viral RNA is converted to DNA and amplified by use of an enzymatic process. The RT-PCR products are analyzed by agarose gel electrophoresis and confirmed by hybridization. The enteric viruses detected by use of this method include enterovirus, hepatitis-A, rotavirus, reovirus, and calicivirus.

For cell-culture analysis, the sample eluate is added to a monlayer of a continuous cell line derived from African green monkey kidney cells (U.S. Environmental Protection Agency, 1996c). Each cell culture is examined microscopically for the appearance of cytopathic effects (CPE) for a total of 14 days; if CPE
is not observed in 14 days, a second passage is done. Results are reported as most probable number of infectious units per volume of water.

QA/QC Activities and Measures
QA/QC activities and measures to take to reduce contamination.
• Use a sterilization indicator, such as autoclave tape, in preparing sample bottles and other equipment
for collection of microbiological samples to determine whether adequate temperatures and pressures have been attained during autoclaving.
• Prepare a separate set of sterile equipment for microbiological sampling at each site.
• Before processing samples in the field vehicle, wipe down the area with a disinfectant (such as isopropyl alcohol) to ensure a sterile working surface.
• Monitor the incubators daily to ensure temperatures are appropriate for the methods used.

For bacteria samples, membrane-filtration (MF) equipment and MF procedure blanks are used to estimate analytical bias.

Field personnel should do the following:
• Prepare an MF equipment blank, a 50- to 100-mL aliquot of sterile buffered water plated before the sample—for every sample by field personnel for total coliform, *E. coli*, and enterococci analyses to determine the sterility of equipment and supplies.
• Prepare a MF procedure blank, a 50- to 100-mL aliquot of sterile buffered water plated after the sample— for every fourth sample to measure the effectiveness of the analyst's rinsing technique or presence of incidental contamination of the buffered water.

If contamination from a MF equipment or procedure blank is found, results are suspect and are qualified or not reported. Proper and consistent procedures for counting and identifying target colonies will be followed, as described in Myers and Sylvester (1997).
• After counting, turn the plate 180° and ensure the second count is within 5 percent of the first count. Have a second analyst check calculations of bacterial concentrations in water for errors.

For coliphage, *Cryptosporidium, Giardia*, and enteric virus samples, equipment and field blanks are used to determine sampling and analytical bias. Equipment blanks for these analyses are different from the MF equipment blanks for bacterial analysis. An equipment blank is a blank solution (sterile buffered water) subjected to the same aspects of sample collection, processing, storage, transportation, and laboratory handling as an environmental sample, but it is processed in an office or laboratory. Field blanks are the same as equipment blanks except that they are generated under actual field conditions.

• For enteric virus analysis, collect one equipment blank after collection of the first sample to ensure that equipment cleaning and sterilization techniques are adequate.
• For coliphage, *Cryptosporidium, Giardia*, and enteric virus analyses, collect field blanks periodically.
At a minimum, the number of field blanks should equal 5 percent of the total number of samples collected. Five percent of samples collected for bacterial and viral indicators (total coliforms, *E. coli*, enterococci, *C. perfringens*, and coliphage) should be nested replicate samples to estimate sampling and analytical variability. For streamwater samples, concurrent replicates to estimate sampling variability are collected by alternating subsamples in each vertical between two collection bottles. For ground-water samples, sequential replicates are collected one after another into separate sterile bottles. Concurrent and sequential replicates are then analyzed in duplicate (split replicates) to estimate analytical variability.

• Because of the expense associated with collection and analysis of samples for pathogens (*Cryptosporidium* and enteric viruses), collect only one replicate sample per year at a site wherein detection of pathogens was found in an earlier sample.
To assess analytical bias of the sampling and analytical method, 2 to 5 percent of the samples collected for enteric virus should be field matrix spikes.
• Run all but 10 L of ground water through the 1 MDS filter and collect the remaining 10 L in a carboy. In the laboratory, the poliovirus vaccine will be added to the 10 L and then passed through the same 1MDS filter. Analysis will be done by use of the cell-culture and RT-PCR methods. • All cell-culture positive samples are serotyped to identify or discount laboratory contamination. Because of the variability in the performance of Method 1623 for recovery of *Cryptosporidium* and *Giardia*, each sample will be collected in duplicate—one will be a regular sample and the other a matrix spike. The laboratory will add a known quantity of cysts and oocysts to the matrix spike to determine recovery efficiency, as described in USEPA (1999c).

Quality Assurance and Quality Control in the Laboratory
The following criteria may be used to evaluate each production analytical laboratory: (1) appropriate, approved, and published methods, (2) documented standard operating procedures, (3) approved quality-assurance plan, (4) types and amount of quality-control data fully documented and technical defensible, (5) participation in the standard reference sample project (6) scientific capability of personnel, and (7) appropriate laboratory equipment.

The microbiology laboratories must follow good laboratory practices—cleanliness, safety practices, procedures for media preparation, specifications for reagent water quality—as set forth by American Public Health Association (1998) and Britton and Greeson (1989). Some specific guidelines are listed in the following paragraphs.

Reference cultures are used by the central laboratory to evaluate the performance of the test procedures, including media and reagents. Pure cultures of *E. coli, Enterobacter aerogenes,* and *Streptococcus faecalis* (American Type Culture Collection, Rockville, Md.) are used to ensure that MF culture media and buffered water are performing adequately. A pure culture of *C. perfringens*, isolated from a sewage sample and verified by standard procedures, is used to evaluate the test procedure and each lot of media and reagents.

Because contamination of samples from coliphage during the analytical procedure is highly probable (Francy and others, 2000), a negative control of host and sterile buffered water is run concurrently with each batch of samples. In addition, to ensure that the method is being executed properly, a positive-control sewage sample is run with each batch of samples. A laminar flow safety hood is recommended for processing the samples for coliphage analysis. Alternatively, a separate coliphage room may be established to discourage laboratory contamination during the analytical process. An ultraviolet light is installed and operated for 8 hours every night in the safety hood or coliphage room to reduce contamination.

The laboratory should follow the QA/QC guidelines in Method 1623 (U.S. Environmental Protection Agency, 1999c) for *Cryptosporidium* and *Giardia* and in the cell-culture and RT-PCR analysis for enteric viruses (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997; U.S. Environmental Protection Agency, 1996c).

Mycobacterium

The mycobacteria are a group of slow-growing organisms. The most important is Mycobacterium tuberculosis, the causative organism of tuberculosis, which takes about 4–6 weeks to grow in the diagnostic laboratory. M. tuberculosis is not a waterborne pathogen; there are, however, a number of Mycobacterium species that occur in the environment and can cause disease in humans. Mycobacterium avium and its related species cause an infection of cervical lymph nodes; it occurs in the environment and is most probably accompanied by ingestion or inhalation. M. avium can grow in water to which no additional nutrients have been added; although water treatment processes of coagulation and filtration appear to reduce the numbers, it is not affected by chlorine levels of 1 mg/ml. It is therefore not surprising that these organisms can regrow and colonize domestic water systems. Once ingested, M. avium can colonize the pharynx without causing any disease. The number of cases reported was very low, but patients with HIV/AIDS are very susceptible.

Another species, Mycobacterium xenopi, has been reported as the waterborne cause of spinal infections following a look-back exercise on over 3000 patients who had undergone discectomy operations some years beforehand (Astagneau et al. 2001).

Mycobacterium paratuberculosis causes Johne's disease in cattle. It is a chronic wasting disease with considerable economic consequences. The organism is extremely difficult to culture; when it does grow, it is very slow and dependent on an exogenous source of mycobactin, which is an iron chelating agent produced by all other mycobacteria. Transmission is by either direct or indirect contact with infected animals and occurs mainly through the faecal–oral route. Organisms are ingested in large numbers by young animals when they feed in troughs that have been contaminated by faeces of shedding animals
(Chiodini et al. 1984).

M. paratuberculosis has recently been suggested as a cause of Crohn's disease, a non-specific chronic transmural inflammatory disease of humans that affects the intestinal tract, commonly the ileum. The disease is chronic, debilitating and of a relapsing nature; the symptoms experienced include diarrhea with blood in the stools and abdominal pain. Complications include obstruction, fistulation and abscesses. There have been many bacteria implicated over the years, but no definite etiological agent has been found. It is thought that immunological mechanisms may play an important role.

Molecular techniques have been developed for the diagnosis of M. paratuberculosis infections and applied to human surgically resected tissues. M. paratuberculosis was detected in approximately 30% of samples, but the sets of results from different laboratories have been conflicting. Some studies were unable to detect the organism; in other studies, the organism was detected in a smaller percentage of healthy subjects. In addition, a few Crohn's disease patients have shown clinical remission when treated with anti-tuberculosis drugs. There is therefore much more work to be done to acquire a better understanding. M. paratuberculosis may be present in surface water contaminated by cattle faeces. Routine testing for indicator organisms would detect faecal pollution, and normal water treatment processes of coagulation and filtration are likely to remove mycobacteria. It is unlikely that drinking-water is a major source of M. paratuberculosis, and its association with Crohn's disease is still under investigation.

Burkholderia Pseudomallei
Burkholderia pseudomallei is the cause of melioidosis, an acute pneumonia often followed by systemic infection with later presentations of abscesses. The organism is widespread in the environment and was originally described in Rangoon in patients compromised by severe poverty who had presumably inhaled the organism in dust when sleeping on the ground. It occurs commonly in southeast Asia and has been detected in service personnel repatriated from those areas in the past. It was also investigated as a biological weapon by several nations, to be

released as an aerosol and cause pneumonia infection in those exposed. A recent study in Bologna, Italy, detected B. pseudomallei in 7% of 85 samples of drinking-water collected from public and private buildings. The mean count was 578 cfu/100 ml. The occurrence of the organism was found to correlate with the HPC at 22 and 36 °C (Zanetti et al. 2000).

Francisella Tularensis

Tularaemia is a zoonosis caused by a highly infective and virulent organism Francisella tularensis, which occurs throughout the northern hemisphere but has never been isolated within the United Kingdom. It occurs in a wide range of animal reservoir posts and can be isolated from the environment in water and mud. It is transmitted to humans who come in close contact with the animal reservoir, arthropods that feed on them or debris and dust associated with them. It can also be transmitted through the ingestion of contaminated water. Human epidemics sometimes occur and are associated with epizootics in the animal

Bacteria of potential health concern 75 populations, evidenced by die-offs. There are several presentations of tularaemia in humans, depending on the route of exposure. Ingestion usually results in

oropharyngeal tularaemia, with fever, pharyngitis and cervical lymphadenitis. Other forms include ulcero-glandular, pleuropneumonic and typhoidal. Following the recent war in Kosovo, over 900 suspected cases of tularaemia were identified and 327 cases confirmed serologically. The epidemiological investigation pointed to rodent-contaminated wells, and rodent carcasses found in some wells tested positive for F. tularensis (Reintjes et al. 2002). In a waterborne outbreak reported from Spain, 19 cases who had contact with river-caught crayfish were identified (Anda et al. 2001). Attempts to isolate F. tularensis from water were unsuccessful. Drinking-water was not involved. F. tularensis is notoriously difficult to culture, requiring a source of cysteine. F. tularensis was investigated and developed as a biological weapon; the infectious dose was found to be extremely low — 10 organisms.

Examples of classical biological warfare agents

Agent	**Disease**
Bacillus anthracis	Anthrax
Brucella species	Brucellosis
Burkholderia mallei	Glanders
Burkholderia pseudomallei	Melioidosis
Francisella tularensis	Tularaemia
Yersinia pestis	Plague
Rickettsia species	Typhus
Coxiella burnetii	Q fever
Clostridium botulinum toxin	Botulism
Staphylococcus aureus enterotoxin B	Staphylococcal food poisoning
Smallpox virus	Smallpox

The remaining bacteria of concern are either heterotrophs that might have a role in disease or emerging pathogens that do have a role in disease and could possibly be waterborne. It is important that these organisms and diseases are kept under surveillance in order to confirm or

refute the suggested associations. Many of the organisms are difficult to grow, and there is no validated trigger of when to look for them.

The HPC does not measure all organisms present, of which many will be non-culturable but viable, and indeed several of the organisms of concern described above would not grow on HPC media. The HPC, however, does give an indication of change in the flora of drinking-water, and the HPC should be evaluated as a trigger for further investigation.

Many new molecular techniques for the detection of pathogens and putative pathogens in water are being described (Waage et al. 1999a,b,c; Lightfoot et al. 2001). DNA chips that have the capacity to detect up to 44 pathogens on one single chip are being developed.

These tests are very expensive when compared with the routine monitoring tests carried out in the water industry and in public health monitoring. The HPC should be evaluated as the signal of changing events in a drinking-water supply to trigger the utilization of these new molecular tests to detect the new bacteria of concern and any associated virulence genes.

Understanding Heterotrophic Plate Count
The analytical methods promulgated under the authority of Section 304(h) of the Clean Water Act are sometimes referred to as the "304(h)" or "Part 136" methods. The methods measure chemical and biological pollutants in media, such as wastewater, ambient water, sediment, and biosolids (sewage sludge). These various CWA methods are tested in a variety of labs and matrices. In addition to Part 136 methods, some approved methods are published or incorporated by reference at 40 CFR Parts 401-503, approved industry-specific methods.

In addition to general purposes methods, EPA has approved special purpose analytical methods. These methods were developed to work in samples or for pollutants specific to certain industrial categories. For example, methods specific to the Pharmaceutical Manufacturing and Pesticide Chemicals industrial categories are listed in Tables IF and IG, respectively, at 40 CFR Part 136.

Methods specific to other industrial categories are listed or incorporated by reference into the regulations at 40 CFR Parts 401-503. These include methods specific to the Pulp, Paper, and Paperboard category (40 CFR Part 430), and specific to Use or Disposal of Sewage Sludge (biosolids) (at 40 CFR Part 503.) Industry-specific methods that are approved for compliance monitoring in the industry for which they are designated may be used for general use, if the same method is listed in Tables IA to IE, or IH at 40 CFR 136.3.

Understanding Total Coliforms
The Total Coliform Rule was published in 1989 and became effective in 1990. The rule set both health goals (MCLGs) and legal limits (MCLs) for the presence of total coliform in drinking water. The rule also detailed the type and frequency of testing that water systems must undertake. In 2003, EPA announced its intent to revise the Total Coliform Rule.

Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration IMS/FA

1.0 Scope and Application

1.1 This method is for determination of the identity and concentration of *Cryptosporidium* (CAS Registry number 137259-50-8) and *Giardia* (CAS Registry number 137259-49-5) in water by filtration, immunomagnetic separation (IMS), and immunofluorescence assay (FA) microscopy. *Cryptosporidium* and *Giardia* may be confirmed using 4',6-diamidino-2-phenylindole (DAPI) staining and differential interference contrast (DIC) microscopy. The method has been validated in surface water, but may be used in other waters, provided the laboratory demonstrates that the method's performance acceptance criteria are met.

1.2 This method is designed to meet the survey and monitoring requirements of the U.S. Environmental Protection Agency (EPA). It is based on laboratory testing of recommendations by a panel of experts convened by EPA. The panel was charged with recommending an improved protocol for recovery and detection of protozoa that could be tested and implemented with minimal additional research.

1.3 This method will not identify the species of *Cryptosporidium* or *Giardia* or the host species of origin, nor can it determine the viability or infectivity of detected oocysts and cysts.

1.4 This method is for use only by persons experienced in the determination of *Cryptosporidium* and *Giardia* by filtration, IMS, and FA. Experienced persons are defined in Section 22.2 as analysts. Laboratories unfamiliar with analyses of environmental samples by the techniques in this method should gain experience using water filtration techniques, IMS, fluorescent antibody staining with monoclonal antibodies, and microscopic examination of biological particulates using bright-field and DIC microscopy.

1.5 Any modification of the method beyond those expressly permitted is subject to the application and approval of alternative test procedures under 40 *CFR* Part 141.27.

2.0 Summary of Method

2.1 A water sample is filtered and the oocysts, cysts, and extraneous materials are retained on the filter. Although EPA has only validated the method using laboratory filtration of bulk water samples shipped from the field, field-filtration also can be used.

2.2 Elution and separation

2.2.1 Materials on the filter are eluted and the eluate is centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated.

2.2.2 The oocysts and cysts are magnetized by attachment of magnetic beads conjugated to anti-*Cryptosporidium* and anti-*Giardia* antibodies. The magnetized oocysts and cysts are separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts.

2.3 Enumeration

2.3.1 The oocysts and cysts are stained on well slides with fluorescently labeled monoclonal antibodies and 4',6-diamidino-2-phenylindole (DAPI). The stained sample is examined using fluorescence and differential interference contrast (DIC) microscopy.

2.3.2 Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of Cryptosporidium oocysts or Giardia cysts. Potential oocysts or cysts are confirmed through DAPI staining characteristics and DIC microscopy. Oocysts and cysts are identified when the size, shape, color, and morphology agree with specified criteria and examples in a photographic library.

2.3.3 Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts or cysts.

2.4 Quality is assured through reproducible calibration and testing of the filtration, immunomagnetic separation (IMS), staining, and microscopy systems. Detailed information on these tests is provided in Section 9.0.

3.0 Definitions

3.1 *Cryptosporidium* is defined as a protozoan parasite potentially found in water and other media. The six species of *Cryptosporidium* and their potential hosts are *C. parvum* (mammals, including humans); *C. baileyi* and *C. meleagridis* (birds); *C. muris* (rodents); *C. serpentis* (reptiles); and *C. nasorum* (fish).

3.2 *Giardia* is defined as a protozoan parasite potentially found in water and other media. The two species of *Giardia* and their potential hosts are *G. intestinalis* (humans) and *G. muris* (mice).

3.3 Definitions for other terms used in this method are given in the glossary (Section 22.0).

4.0 Contamination, Interferences, and Organism Degradation

4.1 Turbidity caused by inorganic and organic debris can interfere with the concentration, separation, and examination of the sample for *Cryptosporidium* oocysts and *Giardia* cysts. In addition to naturally-occurring debris, such as clays and algae, chemicals, such as iron and alum coagulants and polymers, may be added to finished waters during the treatment process, which may result in additional interference.

4.2 Organisms and debris that autofluoresce or demonstrate non-specific fluorescence, such as algal and yeast cells, when examined by epifluorescent microscopy, may interfere with the detection of oocysts and cysts and contribute to false positives by immunofluorescence assay (FA).

4.3 Solvents, reagents, labware, and other sample-processing hardware may yield artifacts that may cause misinterpretation of microscopic examinations for oocysts and cysts. All materials used shall be demonstrated to be free from interferences under the conditions of analysis by running a method blank (negative control sample) initially and a minimum of every week or after changes in source of reagent water. Specific selection of reagents and purification of solvents and other materials may be required.

4.4 Interferences co-extracted from samples will vary considerably from source to source, depending on the water being sampled. Experience suggests that high levels of algae, bacteria, and other protozoa can interfere in the identification of oocysts and cysts (Reference 20.1).

4.5 Freezing samples, filters, eluates, concentrates, or slides may interfere with the detection and/or identification of oocysts and cysts.

4.6 All equipment should be cleaned according to manufacturers' instructions. Disposable supplies should be used wherever possible.

5.0 Safety

5.1 The biohazard associated with, and the risk of infection from, oocysts and cysts is high in this method because live organisms are handled. This method does not purport to address all of the safety problems associated with its use. It is the responsibility of the laboratory to establish appropriate safety and health practices prior to use of this method. In particular, laboratory staff must know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms while preparing, using, and disposing of sample concentrates, reagents and materials, and while operating sterilization equipment.

5.2 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 20.2 through 20.5.

5.3 Samples may contain high concentrations of biohazards and toxic compounds, and must be handled with gloves and opened in a biological safety cabinet to prevent exposure. Reference materials and standards containing oocysts and cysts must also be handled with gloves and laboratory staff must never place gloves in or near the face after exposure to solutions known or suspected to contain oocysts and cysts. Do not mouth-pipette.

5.4 Laboratory personnel must change gloves after handling filters and other contaminant-prone equipment and reagents. Gloves must be removed or changed before touching any other laboratory surfaces or equipment.
5.5 Centers for Disease Control (CDC) regulations (42 CFR 72) prohibit interstate shipment of more than 4 L of solution known to contain infectious materials. State regulations may contain similar regulations for intrastate commerce. Unless the sample is known or suspected to contain *Cryptosporidium*, *Giardia*, or other infectious agents (e.g., during an outbreak), samples should be shipped as noninfectious and should not be marked as infectious. If a sample is known or suspected to be infectious, and the sample must be shipped to a laboratory by a transportation means affected by CDC or state regulations, the sample should be shipped in accordance with these regulations.

6.0 Equipment and Supplies

NOTE*: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 Sample collection equipment for shipment of bulk water samples for laboratory filtration. Collapsible LDPE cubitainer for collection of 10-L bulk sample(s)—Cole Parmer cat. no. U-06100-30 or equivalent. Fill completely to ensure collection of a full 10-L sample. Discard after one use.
6.2 Equipment for sample filtration. Three options have been demonstrated to be acceptable for use with Method 1623. Other options may be used if their acceptability is demonstrated according to the procedures outlined in Section 9.1.2.
6.2.1 Cubitainer spigot to facilitate laboratory filtration of sample (for use with any filtration option)—Cole Parmer cat. no. U-06061-01, or equivalent.
6.2.2 Envirochek™ sampling capsule equipment requirements for use with the procedure described in Section 12.0. The version of the method using this filter was validated using 10-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and source water samples (Section 9.1.2).

6.2.2.1 Sampling capsule—Envirochek™, Pall Gelman Laboratory, Ann Arbor, MI, product 12110
6.2.2.2 Laboratory shaker with arms for agitation of sampling capsules
6.2.2.2.1 Laboratory shaker—Lab-Line model 3589, VWR Scientific cat. no. 57039-055, Fisher cat. no. 14260-11, or equivalent
6.2.2.2.2 Side arms for laboratory shaker—Lab-Line Model 3587-4, VWR Scientific cat. no. 57039-045, Fisher cat. no. 14260-13, or equivalent
6.2.3 CrypTest™ capsule filter equipment requirements. Follow the manufacturer's instructions when using this filtration option. The version of the method using this filter was validated using 10-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and matrix samples (Section 9.1.2).
6.2.3.1 Capsule filter—CrypTest™, Whatman Inc, Clifton, NJ, product no. 610064
6.2.3.2 Cartridge housing—Ametek 5-in. clear polycarbonate, Whatman cat. no. 71503, or equivalent
6.2.3.3 Ultrasonic bath—VWR Model 75T#21811-808, or equivalent
6.2.3.4 Laboratory tubing—Tygon formula R-3603, or equivalent

6.2.4 Filta-Max™ foam filter equipment requirements. Follow the manufacturer's instructions when using this filtration option. The version of the method using this filter was validated using 50-L sample volumes; alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and matrix samples (Section 9.1.2).

6.2.4.1 Foam filter—Filta-Max™, IDEXX, Westbrook, ME. Filter module and membrane: product code FMC 10601; filter membranes (100 pack), product code FMC 10800

NOTE: Check at least one filter per batch to ensure that the filters have not been affected by improper storage or other factors that could result in brittleness or other problems. At a minimum confirm that the test filter expands properly in water before using the batch or shipping filters to the field.

6.2.4.2 Filter processing equipment—Filta-Max starter kit, IDEXX, Westbrook, ME, cat. no. FMC 11002. Includes all equipment required to run and process Filta-Max filter modules (manual wash station (FMC 10102) including plunger head (FMC 12001), elution tubing set (FMC 10301), vacuum set (FMC 10401), filter housing (FMC 10501), and magnetic stirrer (FMC 10901).

6.3 Ancillary sampling equipment

6.3.1 Tubing—Glass, polytetrafluoroethylene (PTFE), high-density polyethylene (HDPE), or other tubing to which oocysts and cysts will not easily adhere—Tygon formula R-3603, or equivalent. If rigid tubing (glass, PTFE, HDPE) is used and the sampling system uses a peristaltic pump, a minimum length of compressible tubing may be used in the pump. Before use, the tubing must be autoclaved, thoroughly rinsed with detergent solution, followed by repeated rinsing with reagent water to minimize sample contamination. Alternately, decontaminate using hypochlorite solution, sodium thiosulfate, and multiple reagent water rinses; dispose of tubing when wear is evident. Dispose of tubing after one use whenever possible.

6.3.2 Flow control valve—0.5 gpm (0.03 L/s), Bertram Controls, Plast-O-Matic cat. no. FC050B½-PV, or equivalent; or 0.4- to 4-Lpm flow meter with valve—Alamo Water Treatment, San Antonio, TX, cat. no. R5310, or equivalent.

6.3.3 Centrifugal pump—Grainger, Springfield, VA, cat. no. 2P613, or equivalent

6.3.4 Flow meter—Sameco cold water totalizer, E. Clark and Associates, Northboro, MA, product no. WFU 10.110, or equivalent.

6.4 Equipment for spiking samples in the laboratory

6.4.1 10-L carboy with bottom delivery port (½")—Cole-Palmer cat. no. 06080-42, or equivalent; calibrate to 10.0 L and mark level with waterproof marker.

6.4.2 Stir bar—Fisher cat. no. 14-511-93, or equivalent.

6.4.3 Stir plate—Fisher cat. no. 14-493-120S, or equivalent.

6.4.4 Hemacytometer—Neubauer type, Hauser Scientific, Horsham, PA, cat. no. 3200 or 1475, or equivalent.

6.4.5 Hemacytometer coverslip—Hauser Scientific, cat. no. 5000 (for hemacytometer cat. no. 3200) or 1461 (for hemacytometer cat. no 1475), or equivalent.

6.4.6 Lens paper without silicone—Fisher cat. no. 11-995, or equivalent.

6.4.7 Polystyrene or polypropylene conical tubes with screw caps—15- and 50-mL.

6.4.8 Equipment required for enumeration of spiking suspensions using membrane filters.

6.4.8.1 Glass microanalysis filter holder—25-mm-diameter, with fritted glass support, Fisher cat. no. 09-753E, or equivalent. Replace stopper with size 8, one-hole rubber stopper, Fisher Cat. No. 14-135M, or equivalent.

6.4.8.2 Three-port vacuum filtration manifold and vacuum source—Fisher Cat. No. 09-753-39A, or equivalent.

6.4.8.3 Cellulose acetate support membrane—1.2-µm-pore-size, 25-mm-diameter, Fisher cat. no. A12SP02500, or equivalent.

6.4.8.4 Polycarbonate track-etch hydrophilic membrane filter—1-µm-pore-size, 25-mm-diameter, Fisher cat. no. K10CP02500, or equivalent.

6.4.8.5 100 × 15 mm polystyrene Petri dishes (bottoms only).

6.4.8.6 60 × 15 mm polystyrene Petri dishes.

6.4.8.7 Glass microscope slides—1 in. × 3 in or 2 in. × 3 in.

6.4.8.8 Coverslips—25 mm^2

6.5 Immunomagnetic separation (IMS) apparatus
6.5.1 Sample mixer—Dynal Inc., Lake Success, NY, cat. no. 947.01, or equivalent.
6.5.2 Magnetic particle concentrator for 10-mL test tubes—Dynal MPC-1® , cat. no. 120.01, or equivalent.
6.5.3 Magnetic particle concentrator for microcentrifuge tubes—Dynal MPC-M®, cat. no. 120.09, or equivalent.
6.5.4 Flat-sided sample tubes—16 × 125 mm Leighton-type tubes with 60 × 10 mm flat-sided magnetic capture area, Dynal L10, cat. no. 740.03, or equivalent.
6.6 Powder-free latex gloves—Fisher cat no. 113945B, or equivalent.
6.7 Graduated cylinders, autoclavable—10-, 100-, and 1000-mL.

6.8 Centrifuges

6.8.1 Centrifuge capable of accepting 15- to 250-mL conical centrifuge tubes and achieving 1500 × G—International Equipment Company, Needham Heights, MA, Centrifuge Size 2, Model K with swinging bucket, or equivalent.
6.8.2 Centrifuge tubes—Conical, graduated, 1.5-, 50-, and 250-mL.

6.9 Microscope

6.9.1 Epifluorescence/differential interference contrast (DIC) with stage and ocular micrometers and 20X (N.A.=0.4) to 100X (N.A.=1.3) objectives—Zeiss™ Axioskop, Olympus™ BH, or equivalent.
6.9.2 Excitation/band-pass filters for immunofluorescence assay (FA)—Zeiss™ 487909 or equivalent, including, 450- to 490-nm exciter filter, 510-nm dicroic beam-splitting mirror, and 515- to 520-nm barrier or suppression filter.
6.9.3 Excitation/band-pass filters for DAPI—Filters cited below (Chroma Technology, Brattleboro, VT), or equivalent.

Microscope model	Fluoro-chrome	Excitation filter (nm)	Dichroic beam-splitting mirror (nm)	Barrier or suppression filter (nm)	Chroma catalog number
Zeiss™ - Axioskop	DAPI (UV)	340-380	400	420	CZ902
Zeiss™ -IM35	DAPI (UV)	340-380	400	420	CZ702
Olympus™ BH	DAPI (UV)	340-380	400	420	11000
			Filter holder		91002
Olympus™ BX	DAPI (UV)	340-380	400	420	11000
			Filter holder		91008
Olympus™ IMT2	DAPI (UV)	340-380	400	420	11000
			Filter holder		91003

6.10 Ancillary equipment for microscopy

6.10.1 Well slides— Spot-On well slides, Dynal cat. no. 740.04; treated, 12-mm diameter well slides, Meridian Diagnostics Inc., Cincinnati, OH, cat. no. R2206; or equivalent.
6.10.2 Glass coverslips—22 × 50 mm.
6.10.3 Nonfluorescing immersion oil.
6.10.4 Micropipette, adjustable: 0- to 10-µL with 0- to 10-µL tips 10- to 100-µL, with 10- to 200-µL tips 100- to 1000-µL with 100- to 1000-µL tips
6.10.5 Forceps—Splinter, fine tip.
6.10.6 Forceps—Blunt-end.
6.10.7 Desiccant—Drierite™ Absorbent, Fisher cat. no. 07-577-1A, or equivalent
6.10.8 Humid chamber—A tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

6.11 Pipettes—Glass or plastic

6.11.1 5-, 10-, and 25-mL.
6.11.2 Pasteur, disposable.

6.12 Balances

6.12.1 Analytical—Capable of weighing 0.1 mg.
6.12.2 Top loading—Capable of weighing 10 mg.

6.13 pH meter

6.14 Incubator—Fisher Scientific Isotemp™, or equivalent.

6.15 Vortex mixer—Fisons Whirlmixer, or equivalent.

6.16 Vacuum source—Capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge.

6.17 Miscellaneous labware and supplies

6.17.1 Test tubes and rack.
6.17.2 Flasks—Suction, Erlenmeyer, and volumetric, various sizes.
6.17.3 Beakers—Glass or plastic, 5-, 10-, 50-, 100-, 500-, 1000-, and 2000-mL.
6.17.4 Lint-free tissues.
6.18 10- to 15-L graduated container—Fisher cat. no. 02-961-50B, or equivalent; calibrate to 9.0, 9.5, 10.0, 10.5, and 11.0 L and mark levels with waterproof marker.
6.19 Filters for filter-sterilizing reagents—Sterile Acrodisc, 0.45 µm , Gelman Sciences cat no. 4184, or equivalent.

7.0 Reagents and Standards

7.1 Reagents for adjusting pH
7.1.1 Sodium hydroxide (NaOH)—ACS reagent grade, 6.0 N and 1.0 N in reagent water 7.1.2 Hydrochloric acid (HCl)—ACS reagent grade, 6.0 N, 1.0 N, and 0.1 N in reagent water.

NOTE: Due to the low volumes of pH-adjusting reagents used in this method, and the impact that changes in pH have on the immunofluorescence assay, the laboratory should purchase standards at the required normality directly from a vendor. Normality should not be adjusted by the laboratory.

7.2 Solvents—Acetone, glycerol, ethanol, and methanol, ACS reagent grade

7.3 Reagent water—Water in which oocysts and cysts and interfering materials and substances, including magnetic minerals, are not detected by this method.

7.4 Reagents for eluting filters

7.4.1 Reagents for eluting Envirochek™ sampling capsules (Section 6.2.2)

7.4.1.1 Laureth-12—PPG Industries, Gurnee, IL, cat. no. 06194, or equivalent. Store Laureth-12 as a 10% solution in reagent water. Weigh 10 g of Laureth-12 and dissolve using a microwave or hot plate in 90 mL of reagent water. Dispense 10-mL aliquots into sterile vials and store at room temperature for up to 2 months, or in the freezer for up to a year.

7.4.1.2 1 M Tris, pH 7.4—Dissolve 121.1 g Tris (Fisher cat. no. BP152) in 700 mL of reagent water and adjust pH to 7.4 with 1 N HCl or NaOH. Dilute to a final 1000 mL with reagent water and adjust the final pH. Filter-sterilize through a 0.2-µm membrane into a sterile plastic container and store at room temperature.

7.4.1.3 0.5 M EDTA, 2 Na, pH 8.0—Dissolve 186.1 g ethylenediamine tetraacetic acid, disodium salt dihydrate (Fisher cat. no. S311) in 800 mL and adjust pH to 8.0 with 6.0 N HCl or NaOH. Dilute to a final volume of 1000 mL with reagent water and adjust to pH 8.0 with 1.0 N HCl or NaOH.

7.4.1.4 Antifoam A—Sigma Chemical Co. cat. no. A5758, or equivalent

7.4.1.5 Preparation of elution buffer solution—Add the contents of a pre-prepared Laureth-12 vial (Section 7.4.1.1) to a 1000-mL graduated cylinder. Rinse the vial several times to ensure the transfer of the detergent to the cylinder. Add 10 mL of Tris solution (Section 7.4.1.2), 2 mL of EDTA solution (Section 7.4.1.3), and 150 µL Antifoam A (Section 7.4.1.4). Dilute to 1000 mL with reagent water.

7.4.2 Reagents for eluting CrypTest™ capsule filters (Section 6.2.3). To 900 mL of reagent water add 8.0 g NaCl, 0.2 g KH_2PO_4, 2.9 g Na_2HPO_4 ($12H_2O$) 0.2 g KCl, 0.2 g sodium lauryl sulfate (SDS), 0.2 mL Tween 80, and 0.02 mL Antifoam A (Sigma Chemical Co. cat. no. A5758, or equivalent). Adjust volume to 1 L with reagent water and adjust pH to 7.4 with 1 N NaOH or HCl.

7.4.3 Reagents for eluting Filta-Max™ foam filters (Section 6.2.4)

7.4.3.1 Phosphate buffered saline (PBS), pH 7.4—Sigma Chemical Co. cat. no. P-3813, or equivalent. Alternately, prepare PBS by adding the following to 1 L of reagent water: 8 g NaCl; 0.2 g KCl; 1.15 g Na_2HPO_4, anhydrous; and 0.2 g KH_2PO_4.

7.4.3.2 Tween 20—Sigma Chemical Co. cat. no. P-7949, or equivalent.

7.4.3.3 High-vacuum grease—BDH/Merck. cat. no. 636082B, or equivalent.

7.4.3.4 Preparation of PBST elution buffer. Add the contents of one sachet of PBS to 1.0 L of reagent water. Dissolve by stirring for 30 minutes. Add 100 µL of Tween 20. Mix by stirring for 5 minutes.

7.5 Reagents for immunomagnetic separation (IMS)—Dynabeads® GC-Combo, Dynal cat. nos. 730.02, 730.12, or equivalent.

7.6 Direct antibody labeling reagents for detection of oocysts and cysts. Store reagents at 0 ^{o}C to 8 ^{o}C and return promptly to this temperature after each use. Do not allow any of the reagents to freeze. The reagents should be protected from exposure to light. Diluted, unused working reagents should be discarded after 48 hours. Discard reagents after the expiration date is reached. The labeling reagents in Sections 7.6.1-7.6.3 have been approved for use with this method.

7.6.1 Merifluor Cryptosporidium/Giardia, Meridian Diagnostics cat. no. 250050, Cincinnati, OH, or equivalent.

7.6.2 Aqua-Glo™ G/C Direct FL, Waterborne cat. no. A100FLR, New Orleans, LA, or equivalent.

7.6.3 Crypt-a-Glo™ and Giardi-a-Glo™, Waterborne cat. nos. A400FLR and A300FLR, respectively, New Orleans, LA, or equivalent.

NOTE: If a laboratory will use multiple types of labeling reagents, the laboratory must demonstrate acceptable performance through an initial precision and recovery test (Section 9.4) for each type, and must perform positive and negative staining controls for each batch of slides stained using each product. However, the laboratory is not required

to analyze additional ongoing precision and recovery samples or method blank samples for each type.

7.6.4 Diluent for labeling reagents—Phosphate buffered saline (PBS), pH 7.4—Sigma Chemical Co. cat. no. P-3813, or equivalent. Alternately, prepare PBS by adding the following to 1 L of reagent water: 8 g NaCl; 0.2 g KCl; 1.15 g Na_2HPO_4, anhydrous; and 0.2 g KH_2PO_4. Filter-sterilize (Section 6.19) or autoclave. Discard if growth is detected or after 6 months, whichever comes first.

7.7 4',6-diamidino-2-phenylindole (DAPI) stain—Sigma Chemical Co. cat. no. A5758, or equivalent.

7.7.1 Stock solution—Dissolve 2 mg/mL DAPI in absolute methanol. Prepare volume consistent with minimum use. Store at 0 °C to 8 °C in the dark. Do not allow to freeze. Discard unused solution when positive staining control fails.

7.7.2 Staining solution (1/5000 dilution in PBS [Section 7.6.4])—Add 10 µL of 2 mg/mL DAPI stock solution to 50 mL of PBS. Prepare daily. Store at 0 °C to 8 °C in the dark except when staining. Do not allow to freeze. The solution concentration may be increased up to 1µg /mL if fading/diffusion of DAPI staining is encountered, but the staining solution must be tested first on expendable environmental samples to confirm that staining intensity is appropriate.

7.8 Mounting medium

7.8.1 DABCO/glycerol mounting medium (2%)—Dissolve 2 g of DABCO (Sigma Chemical Co. cat no. D-2522, or equivalent) in 95 mL of warm glycerol/PBS (60% glycerol, 40% PBS [Section 7.6.4]). After the DABCO has dissolved completely, adjust the solution volume to 100 mL by adding an appropriate volume of glycerol/PBS solution. Alternately, dissolve the DABCO in 40 mL of PBS, then add azide (1 mL of 100X, or 10% solution), then 60 mL of glycerol.

7.8.2 Mounting medium supplied with Merifluor direct labeling kit (Section 7.6.1)

7.9 Clear fingernail polish or clear fixative, PGC Scientifics, Gaithersburg, MD, cat. no. 60-4890, or equivalent.

7.10 Oocyst and cyst suspensions for spiking

7.10.1 Enumerated spiking suspensions prepared by flow cytometer—not heat-fixed or formalin fixed: Wisconsin State Laboratory of Hygiene Flow Cytometry Unit or equivalent

7.10.2 Materials for manual enumeration of spiking suspensions

7.10.2.1 Purified Cryptosporidium oocyst stock suspension for manual enumeration—not heat-fixed or formalin-fixed: Sterling Parasitology Laboratory, University of Arizona, Tucson, or equivalent

7.10.2.2 Purified Giardia cyst stock suspension for manual enumeration—not heat-fixed or formalin-fixed: Waterborne, Inc., New Orleans, LA; Hyperion Research, Medicine Hat, Alberta, Canada; or equivalent

7.10.2.3 Tween-20, 0.01%—Dissolve 1.0 mL of a 10% solution of Tween-20 in 1 L of reagent water

7.10.2.4 Storage procedure—Store oocyst and cyst suspensions at 0 °C to 8 °C, until ready to use; do not allow to freeze

7.11 Additional reagents for enumeration of spiking suspensions using membrane filtration (Section 11.3.6)—Sigmacote® Sigma Company Product No. SL-2, or equivalent

8.0 Sample Collection and Storage

8.1 Samples are collected as bulk samples and shipped to the laboratory for processing through the entire method, or are filtered in the field and shipped to the laboratory for processing from elution (Section 12.2.6) onward. Samples must be shipped via overnight service on the day they are collected. Chill samples as much as possible between collection and shipment by storing in a refrigerator or pre-icing the sample in a cooler. If the sample is pre-iced before shipping, replace with fresh ice immediately before shipment. Samples should be shipped at 0 °C to 8 °C, unless the time required to chill the sample to 8 °C would prevent the sample from being shipped

overnight for receipt at the laboratory the day after collection. Samples must not be allowed to freeze. Upon receipt, the laboratory should record the temperature of the samples and store them refrigerated at 0 ^{o}C to 8 ^{o}C until processed. Results from samples shipped overnight to the laboratory and received at >8 ^{o}C should be qualified by the laboratory.

NOTE: See transportation precautions in Section 5.5.

8.2 Sample holding times. Sample processing should be completed as soon as possible by the laboratory. The laboratory should complete sample filtration, elution, concentration, purification, and staining the day the sample is received wherever possible. However, the laboratory is permitted to split up the sample processing steps if processing a sample completely in one day is not possible. If this is necessary, sample processing can be halted after filtration, application of the purified sample onto the slide, or staining. Table 1, in Section 21.0 provides a breakdown of the holding times for each set of steps. Sections 8.2.1 through 8.2.4 provide descriptions of these holding times.
8.2.1 Sample collection and filtration. Sample elution must be initiated within 96 hours of sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field).
8.2.2 Sample elution, concentration, and purification. The laboratory must complete the elution, concentration, and purification (Sections 12.2.6 through 13.3.3.11) in one work day. It is critical that these steps be completed in one work day to minimize the time that any target organisms present in the sample sit in eluate or concentrated matrix. This process ends with the application of the purified sample on the slide for drying.
8.2.3 Staining. The sample must be stained within 72 hours of application of the purified sample to the slide.
8.2.4 Examination. Although immunofluorescence assay (FA) and 4',6-diamidino-2-phenylindole (DAPI) and differential interference contrast (DIC) microscopy examination and confirmation should be performed immediately after staining is complete, laboratories have up to 7 days from completion of sample staining to complete the examination and confirmation of samples. However, if fading/diffusion of FITC or DAPI staining is noticed, the laboratory must reduce this holding time. In
addition the laboratory may adjust the concentration of the DAPI staining solution (Sections 7.7.2) so that fading/diffusion does not occur.
8.5 Spiking suspension enumeration holding times. Flow-cytometer-sorted spiking suspensions (Sections 7.10.1 and 11.2) used for spiked quality control (QC) samples (Section 9) must be used within the expiration date noted on the suspension. Laboratories should use flow-cytometer sorted spiking suspensions containing live organisms within two weeks of preparation at the flow cytometry laboratory. Manually enumerated spiking suspensions must be used within 24 hours of enumeration of the spiking suspension if the hemacytometer chamber technique is used (Section 11.3.4); or within 24 hours of application of the spiking suspension to the slides if the well slide or membrane filter enumeration technique is used (Sections 11.3.5 and 11.3.6).

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance (QA) program (Reference 20.6). The minimum requirements of this program consist of an initial demonstration of laboratory capability through performance of the initial precision and recovery (IPR) test (Section 9.4), analysis of spiked samples to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.
9.1.1 A test of the microscope used for detection of oocysts and cysts is performed prior to examination of slides. This test is described in Section 10.0.
9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted to modify certain method procedures to improve recovery or lower the costs of measurements, provided that all required quality control (QC) tests are performed and

all QC acceptance criteria are met. Method procedures that can be modified include front-end techniques, such as filtration or immunomagnetic separation (IMS). The laboratory is not permitted to use an alternate determinative technique to replace immunofluorescence assay in this method (the use of different determinative techniques are considered to be different methods, rather than modified version of this method). However, the laboratory is permitted to modify the immunofluorescence assay procedure, provided that all required QC tests are performed (Section 9.1.2.1) and all QC acceptance criteria are met (see guidance on the use of multiple labeling reagents in Section 7.6).

9.1.2.1 Method modification validation/equivalency demonstration requirements.
9.1.2.1.1 Method modifications at a single laboratory. Each time a modification is made to this method for use in a single laboratory, the laboratory is required to validate the modification according to Tier 1 of EPA's performance-based measurement system (PBMS) (Table 2 and Reference 20.7) to demonstrate that the modification produces results equivalent or superior to results produced by this method as written. Briefly, each time a modification is made to this method, the laboratory is required to demonstrate acceptable modified method performance through the IPR test (Section 9.4). IPR results must meet the QC acceptance criteria in Tables 3 and 4 in Section 21.0, and should be comparable to previous results using the unmodified procedure. Although not required, the laboratory also should perform a matrix spike/matrix spike duplicate (MS/MSD) test to demonstrate the performance of the modified method in at least one real-world matrix before analyzing field samples using the modified method. The laboratory is required to perform MS samples using the modified method at the frequency noted in Section 9.1.8.
9.1.2.1.2 Method modifications for nationwide approval. If the laboratory or a manufacturer seeks EPA approval of a method modification for nationwide use, the laboratory or manufacturer must validate the modification according to Tier 2 of EPA's PBMS (Table 2 and Reference 20.7). Briefly, at least three laboratories must perform IPR tests (Section 9.4) and MS/MSD (Section 9.5) tests using the modified method, and all tests must meet the QC acceptance criteria specified in Tables 3 and 4 in Section 21.0. Upon nationwide approval, laboratories electing to use the modified method still must demonstrate acceptable performance in their own laboratory according to the requirements in Section 9.1.2.1.1.
9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:
9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.
9.1.2.2.2 A listing of the analyte(s) measured (Cryptosporidium and Giardia).
9.1.2.2.3 9.1.2.2.4 A narrative stating reason(s) for the modification.
9.1.2.2.5 Results from all QC tests comparing the modified method to this method, including: (a) IPR (Section 9.4) (b) MS/MSD (Section 9.5) (c) Analysis of method blanks (Section 9.6) Data that will allow an independent reviewer to validate each determination by tracing the following processing and analysis steps leading to the final result:

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the following processing and analysis steps leading to the final result:
(a) Sample numbers and other identifiers
(b) Source of spiking suspensions, as well as lot number and date received (Section 7.10)
(c) Spike enumeration date and time
(d) All spiking suspension enumeration counts and calculations (Section 11.0)
(e) Sample spiking dates and times
(f) Volume filtered (Section 12.2.5.2)
(g) Filtration and elution dates and times
(h) Pellet volume, resuspended concentrate volume, resuspended concentrate volume transferred to IMS, and all calculations required to verify the percent of concentrate examined (Section 13.2)

(i) Purification completion dates and times (Section 3.3.3.11)
(j) Staining completion dates and times (Section 14.10)
(k) Staining control results (Section 15.2.1)
(l) All required examination information (Section 15.2.2)
(m) Examination completion dates and times (Section 15.2.4)
(n) Analysis sequence/run chronology
(o) Lot numbers of elution, IMS, and staining reagents
(p) Copies of bench sheets, logbooks, and other recordings of raw data
(q) Data system outputs, and other data to link the raw data to the results reported

9.1.3 The laboratory shall spike a separate sample aliquot from the same source to monitor method performance. This MS test is described in Section 9.5.1.

9.1.4 Analysis of method blanks is required to demonstrate freedom from contamination. The procedures and criteria for analysis of a method blank are described in Section 9.6.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery (OPR) sample that the analysis system is in control. These procedures are described in Section 9.7.

9.1.6 The laboratory shall maintain records to define the quality of data that are generated. Development of accuracy statements is described in Sections 9.5.1.4 and 9.7.3.

9.1.7 The laboratory shall analyze one method blank (Section 9.6) and one OPR sample (Section 9.7) each week during which samples are analyzed if 20 or fewer field samples are analyzed during this period. The laboratory shall analyze one laboratory blank and one OPR sample for every 20 samples if more than 20 samples are analyzed in a week.

9.1.8 The laboratory shall analyze one MS sample (Section 9.5.1) when samples are first received from a utility for which the laboratory has never before analyzed samples. The MS analysis is performed on an additional (second) sample sent from the utility. If the laboratory routinely analyzes samples from 1 or more utilities, 1 MS analysis must be performed per 20 field samples. For example, when a laboratory receives the first sample from a given site, the laboratory must obtain a second aliquot of this sample to be used for the MS. When the laboratory receives the 21st sample from this site, a separate aliquot of this 21st sample must be collected and spiked.

9.2 Micropipette calibration

9.2.1 Micropipettes must be sent to the manufacturer for calibration annually. Alternately, a qualified independent technician specializing in micropipette calibration can be used. Documentation on the precision of the recalibrated micropipette must be obtained from the manufacturer or technician.

9.2.2 Internal and external calibration records must be kept on file in the laboratory's QA logbook.

9.2.3 If a micropipette calibration problem is suspected, the laboratory shall tare an empty weighing boat on the analytical balance and pipette the following volumes of reagent water into the weigh boat using the pipette in question: 100% of the maximum dispensing capacity of the micropipette, 50% of the capacity, and 10% of the capacity. Ten replicates should be performed at each weight. Record the weight of the water (assume that 1.00 mL of reagent water weighs 1.00 g) and calculate the relative standard deviation (RSD) for each. If the weight of the reagent water is within 1% of the desired weight (mL) and the RSD of the replicates at each weight is within 1%, then the pipette remains acceptable for use.

9.2.4 If the weight of the reagent water is outside the acceptable limits, consult the manufacturer's instruction manual troubleshooting section and repeat steps described in Section 9.2.3. If problems with the pipette persist, the laboratory must send the pipette to the manufacturer for recalibration.

9.3 Microscope adjustment and certification: Adjust the microscope as specified in Section 10.0. All of the requirements in Section 10.0 must be met prior to analysis of IPRs, blanks, OPRs, field samples, and MS/MSDs.

9.4 Initial precision and recovery (IPR)—To establish the ability to demonstrate control over the analytical system and to generate acceptable precision and recovery, the laboratory shall perform the following operations:

9.4.1 Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), spike, filter, elute, concentrate, separate (purify), stain, and examine four reagent water samples spiked with 100 to 500 oocysts and 100 to 500 cysts. If more than one process will be used for filtration and/or separation of samples, a separate set of IPR samples must be prepared for each process.

NOTE: IPR tests must be accompanied by analysis of a method blank (Section 9.6).

9.4.2 Using results of the four analyses, calculate the average percent recovery and the relative standard deviation (RSD) of the recoveries for Cryptosporidium and for Giardia. The RSD is the standard deviation divided by the mean times 100.

9.4.3 Compare RSD and the mean with the corresponding limits for initial precision and recovery in Tables 3 and 4 in Section 21.0. If the RSD and the mean meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If the RSD or the mean falls outside the range for recovery, system performance is unacceptable. In this event, correct the problem and repeat the test (Section 9.4.1).

9.5 Matrix spike (MS) and matrix spike duplicate (MSD):

9.5.1 Matrix spike—The laboratory shall spike and analyze a separate field sample aliquot to determine the effect of the matrix on the method's oocyst and cyst recovery. The MS shall be analyzed according to the frequency in Section 9.1.8.

9.5.1.1 Analyze an unspiked field sample according to the procedures in Sections 12.0 to 15.0. Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), spike, filter, elute, concentrate, separate (purify), stain, and examine a second field sample aliquot with the number of organisms used in the IPR or OPR tests (Sections 9.4 and 9.7).

9.5.1.2 For each organism, calculate the percent recovery (R) using the following equation.

$$R = 100 \times \frac{Nsp - Ns}{T}$$

where

R is the percent recovery

N_{sp} is the number of oocysts or cysts detected in the spiked sample

N_s is the number of oocysts or cysts detected in the unspiked sample T is the true value of the oocysts or cysts spiked

9.5.1.3 Compare the recovery for each organism with the corresponding limits in Tables 3 and 4 in Section 21.0.

NOTE: Some sample matrices may prevent the acceptance criteria in Tables 3 and 4 from being met. An assessment of the distribution of MS recoveries across 430 MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

9.5.1.4 As part of the QA program for the laboratory, method precision for samples should be assessed and records maintained. After the analysis of five samples for which the spike recovery for each organism passes the tests in Section 9.5.1.3, the laboratory should calculate the average percent recovery (P) and the standard deviation of the percent recovery (s_r). Express the precision assessment as a percent recovery interval from P ˉ 2 s_r to P + 2 s_r for each matrix. For example, if P = 80% and s_r = 30%, the accuracy interval is expressed as 20% to 140%. The

precision assessment should be updated regularly across all MS samples and stratified by MS samples for each source.
9.5.2 Matrix spike duplicate—MSD analysis is required as part of nationwide approval of a modified version of this method to demonstrate that the modified version of this method produces results equal or superior to results produced by the method as written (Section 9.1.2.1.2). At the same time the laboratory spikes and analyzes the second field sample aliquot in Section 9.5.1.1, the laboratory shall spike and analyze a third, identical field sample aliquot.

NOTE: Matrix spike duplicate samples are only required for Tier 2 validation studies. They are recommended for Tier 1 validation, but not required.

9.5.2.1 For each organism, calculate the percent recovery (R) using the equation in Section 9.5.1.2.

9.5.2.2 Calculate the mean of the number of oocysts or cysts in the MS and MSD (X_{mean}) (= [MS+MSD]/2).

9.5.2.3 Calculate the relative percent difference (RPD) of the recoveries using the following equation:

$$RPD = 100 \frac{| NMS - NMSD |}{Xmean}$$

where
RPD is the relative percent difference
N_{MS} is the number of oocysts or cysts detected in the MS
N_{MSD} is the number of oocysts or cysts detected in the MSD
X_{mean} is the mean number of oocysts or cysts detected in the MS and MSD

9.5.2.4 Compare the mean MS/MSD recovery and RPD with the corresponding limits in Tables 3 and 4 in Section 21.0 for each organism.

9.6 Method blank (negative control sample, laboratory blank): Reagent water blanks are analyzed to demonstrate freedom from contamination. Analyze the blank immediately prior to analysis of the IPR test (Section 9.4) and OPR test (Section 9.7) and prior to analysis of samples for the week to demonstrate freedom from contamination.
9.6.1 Filter, elute, concentrate, separate (purify), stain, and examine at least one reagent water blank per week (Section 9.1.7) according to the procedures in Sections 12.0 to 15.0. If more than 20 samples are analyzed in a week, process and analyze one reagent water blank for every 20 samples.
9.6.2 If *Cryptosporidium* oocysts, *Giardia* cysts, or any potentially interfering organism or material is found in the blank, analysis of additional samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. Any sample in a batch associated with a contaminated blank that shows the presence of one or more oocysts or cysts is assumed to be contaminated and should be recollected, if possible. Any method blank in which oocysts or cysts are not detected is assumed to be uncontaminated and may be reported.

9.7 Ongoing precision and recovery ([OPR]; positive control sample; laboratory control sample): Using the spiking procedure in Section 11.4 and enumerated spiking suspensions (Section 7.10.1 or Section 11.3), filter, elute, concentrate, separate (purify), stain, and examine at least one reagent water sample spiked with 100 to 500 oocysts and 100 to 500 cysts each week to verify all performance criteria. The laboratory must analyze one OPR sample for every 20 samples if more than 20 samples are analyzed in a week. If multiple method variations are used, separate OPR samples must be prepared for each method variation. Adjustment and/or

recalibration of the analytical system shall be performed until all performance criteria are met. Only after all performance criteria are met may samples be analyzed.
9.7.1 Examine the slide from the OPR prior to analysis of samples from the same batch.
9.7.1.1 Using 200X to 400X magnification, more than 50% of the oocysts or cysts must appear undamaged and morphologically intact; otherwise, the analytical process is damaging the organisms. Determine the step or reagent that is causing damage to the organisms. Correct the problem and repeat the OPR test.

9.7.1.2 Identify and enumerate each organism using epifluorescence microscopy. The first three presumptive *Cryptosporidium* oocysts and three *Giardia* cysts identified in the OPR sample must be examined using FITC, DAPI, and DIC, as per Section 15.2, and the detailed characteristics (size, shape, DAPI category, and DIC category) reported on the *Cryptosporidium* and *Giardia* report form, as well as any additional comments on organism appearance, if notable.

9.7.2 For each organism, calculate the percent recovery (R) using the following equation:

$$R = 100 \times \frac{N}{T}$$

where:
R = the percent recovery
N = the number of oocysts or cysts detected
T = the number of oocysts or cysts spiked

9.7.3 Compare the recovery with the limits for ongoing precision and recovery in Tables 3 and 4 in Section 21.0. If the recovery meets the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, the recovery falls outside of the range given, system performance is unacceptable. In this event, there may be a problem with the microscope or with the filtration or separation systems. Troubleshoot the problem using the procedures at Section 9.7.4 as a guide. After assessing the issue, reanalyze the OPR sample. All samples must be associated with an OPR that passes the criteria in Section 21.0. Samples that are not associated with an acceptable OPR must be flagged accordingly.

9.7.4 Troubleshooting. If an OPR sample has failed, and the cause of the failure is not known, the laboratory generally should identify the problem working backward in the analytical process from the microscopic examination to filtration.
9.7.4.1 Microscope system and antibody stain: To determine if the failure of the OPR test is due to changes in the microscope or problems with the antibody stain, re-examine the positive staining control (Section 15.2.1), check Köhler illumination, and check the fluorescence of the fluorescein-labeled monoclonal antibodies (Mabs) and 4',6-diamidino-2-phenylindole (DAPI). If results are unacceptable, re-examine the previously-prepared positive staining control to determine whether the problem is associated with the microscope or the antibody stain.
9.7.4.2 Separation (purification) system: To determine if the failure of the OPR test is attributable to the separation system, check system performance by spiking a 10-mL volume of reagent water with 100 - 500 oocysts and cysts and processing the sample through the IMS, staining, and examination procedures in Sections 13.3 through 15.0.
9.7.4.3 Filtration/elution/concentration system: If the failure of the OPR test is attributable to the filtration/elution/concentration system, check system performance by processing spiked reagent water according to the procedures in Section 12.2 through 13.2.2.1, and filter, stain, and examine the sample concentrate according to Section 11.3.6.

9.7.5 The laboratory should add results that pass the specifications in Section 9.7.3 to initial and previous ongoing data and update the QC chart to form a graphic representation of continued laboratory performance. The laboratory should develop a statement of laboratory accuracy (reagent water, raw surface water) by calculating the average percent recovery (R) and the

standard deviation of percent recovery (s_r). Express the accuracy as a recovery interval from R ¯2 s_r to R + 2 s_r. For example, if R = 95% and s_r = 25%, the accuracy is 45% to 145%.
9.8 The laboratory should periodically analyze an external QC sample, such as a performance evaluation or standard reference material, when available.

The laboratory also should periodically participate in interlaboratory comparison studies using the method.

9.9 The specifications contained in this method can be met if the analytical system is under control. The standards used for initial (Section 9.4) and ongoing (Section 9.7) precision and recovery should be identical, so that the most precise results will be obtained. The microscope in particular will provide the most reproducible results if dedicated to the settings and conditions required for the determination of Cryptosporidium and Giardia by this method.
9.10 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and duplicate spiked samples may be required to determine the precision of the analysis.

10.0 Microscope Calibration and Analyst Verification
10.1 In a room capable of being darkened to near-complete darkness, assemble the microscope, all filters, and attachments. The microscope should be placed on a solid surface free from vibration. Adequate workspace should be provided on either side of the microscope for taking notes and placement of slides and ancillary materials.
10.2 Using the manuals provided with the microscope, all analysts must familiarize themselves with operation of the microscope.

10.3 Microscope adjustment and calibration (adapted from Reference 20.6)

10.3.1 Preparations for adjustment

10.3.1.1 The microscopy portion of this procedure depends upon proper alignment and adjustment of very sophisticated optics. Without proper alignment and adjustment, the microscope will not function at maximal efficiency, and reliable identification and enumeration of oocysts and cysts will not be possible. Consequently, it is imperative that all portions of the microscope from the light sources to the oculars are properly adjusted.
10.3.1.2 While microscopes from various vendors are configured somewhat differently, they all operate on the same general physical principles. Therefore, slight deviations or adjustments may be required to make the procedures below work for a particular instrument.
10.3.1.3 The sections below assume that the mercury bulb has not exceeded time limits of operation, that the lamp socket is connected to the lamp house, and that the condenser is adjusted to produce Köhler illumination.
10.3.1.4 Persons with astigmatism should always wear contact lenses or glasses when using the microscope.

CAUTION: In the procedures below, do not touch the quartz portion of the mercury bulb with your bare fingers. Finger oils can cause rapid degradation of the quartz and premature failure of the bulb.
WARNING: Never look at the ultraviolet (UV) light from the mercury lamp, lamp house, or the UV image without a barrier filter in place. UV radiation can cause serious eye damage.

10.3.2 Epifluorescent mercury bulb adjustment: The purpose of this procedure is to ensure even field illumination. This procedure must be followed when the microscope is first used, when replacing bulbs, and if problems such as diminished fluorescence or uneven field illumination are experienced.

10.3.2.1 Remove the diffuser lens between the lamp and microscope or swing it out of the transmitted light path.
10.3.2.2 Using a prepared microscope slide, adjust the focus so the image in the oculars is sharply defined.
10.3.2.3 Replace the slide with a business card or a piece of lens paper.
10.3.2.4 Close the field diaphragm (iris diaphragm in the microscope base) so only a small point of light is visible on the card. This dot of light indicates the location of the center of the field of view.
10.3.2.5 Mount the mercury lamp house on the microscope without the UV diffuser lens in place and turn on the mercury bulb.
10.3.2.6 Remove the objective in the light path from the nosepiece. A primary (brighter) and secondary image (dimmer) of the mercury bulb arc should appear on the card after focusing the image with the appropriate adjustment.
10.3.2.7 Using the lamp house adjustments, adjust the primary and secondary mercury bulb images so they are side by side (parallel to each other) with the transmitted light dot in between them.
10.3.2.8 Reattach the objective to the nosepiece.
10.3.2.9 Insert the diffuser lens into the light path between the mercury lamp house and the microscope.
10.3.2.10 Turn off the transmitted light and replace the card with a slide of fluorescent material. Check the field for even fluorescent illumination. Adjustment of the diffuser lens probably will be required. Additional slight adjustments as in Section 10.3.2.7 above may be required.
10.3.2.11 Maintain a log of the number of hours the UV bulb has been used. Never use the bulb for longer than it has been rated. Fifty-watt bulbs should not be used longer than 100 hours; 100-watt bulbs should not be used longer than 200 hours.

10.3.3 Transmitted bulb adjustment: The purpose of this procedure is to center the filament and ensure even field illumination. This procedure must be followed when the bulb is changed.
10.3.3.1 Remove the diffuser lens between the lamp and microscope or swing it out of the transmitted light path.
10.3.3.2 Using a prepared microscope slide and a 40X (or similar) objective, adjust the focus so the image in the oculars is sharply defined.
10.3.3.3 Without the ocular or Bertrand optics in place, view the pupil and filament image at the bottom of the tube.
10.3.3.4 Focus the lamp filament image with the appropriate adjustment on the lamp house.
10.3.3.5 Similarly, center the lamp filament image within the pupil with the appropriate adjustment(s) on the lamp house.
10.3.3.6 Insert the diffuser lens into the light path between the transmitted lamp house and the microscope.

10.3.4 Adjustment of the interpupillary distance and oculars for each eye: These adjustments are necessary so that eye strain is reduced to a minimum, and must be made for each individual using the microscope. Section 10.3.4.2 assumes use of a microscope with both oculars adjustable; Section 10.3.4.3 assumes use of a microscope with a single adjustable ocular. The procedure must be followed each time an analyst uses the microscope.
10.3.4.1 Interpupillary distance

10.3.4.1.1 Place a prepared slide on the microscope stage, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.
10.3.4.1.2 Using both hands, move the oculars closer together or farther apart until a single circle of light is observed while looking through the oculars with both eyes. Note interpupillary distance.

10.3.4.2 Ocular adjustment for microscopes capable of viewing a photographic frame through the viewing binoculars: This procedure assumes both oculars are adjustable.

10.3.4.2.1 Place a card between the right ocular and eye keeping both eyes open. Adjust the correction (focusing) collar on the left ocular by focusing the left ocular until it reads the same as the interpupillary distance. Bring an image located in the center of the field of view into as sharp a focus as possible.
10.3.4.2.2 Transfer the card to between the left eye and ocular. Again keeping both eyes open, bring the same image into as sharp a focus for the right eye as possible by adjusting the ocular correction (focusing) collar at the top of the right ocular.

10.3.4.3 Ocular adjustment for microscopes without binocular capability: This procedure assumes a single focusing ocular. The following procedure assumes that only the right ocular is capable of adjustment.

10.3.4.3.1 Place a card between the right ocular and eye keeping both eyes open. Using the fine adjustment, focus the image for the left eye to its sharpest point.
10.3.4.3.2 Transfer the card to between the left eye and ocular. Keeping both eyes open, bring the image for the right eye into sharp focus by adjusting the ocular collar at the top of the ocular without touching the coarse or fine adjustment.
10.3.5 Calibration of an ocular micrometer: This section assumes that a reticle has been installed in one of the oculars by a microscopy specialist and that a stage micrometer is available for calibrating the ocular micrometer (reticle). Once installed, the ocular reticle should be left in place. The more an ocular is manipulated the greater the probability is for it to become contaminated with dust particles. This calibration should be done for each objective in use on the microscope. If there is a top lens on the microscope, the calibration procedure must be done for the respective objective at each top lens setting. The procedure must be followed when the microscope is first used and each time the objective is changed.
10.3.5.1 Place the stage micrometer on the microscope stage, turn on the transmitted light, and focus the micrometer image using the coarse and fine adjustment knobs for the objective to be calibrated. Continue adjusting the focus on the stage micrometer so you can distinguish between the large (0.1 mm) and the small (0.01 mm) divisions.
10.3.5.2 Adjust the stage and ocular with the micrometer so the 0 line on the ocular micrometer is exactly superimposed on the 0 line on the stage micrometer.
10.3.5.3 Without changing the stage adjustment, find a point as distant as possible from the two 0 lines where two other lines are exactly superimposed.
10.3.5.4 Determine the number of ocular micrometer spaces as well as the number of millimeters on the stage micrometer between the two points of superimposition. For example: Suppose 48 ocular micrometer spaces equal 0.6 mm.
10.3.5.5 Calculate the number of mm/ocular micrometer space. For example:
0.6 *mm* 0.0125 *mm* = 48 *ocular micrometer spaces ocular micrometer space*

10.3.5.6 Because most measurements of microorganisms are given in µm rather than mm, the value calculated above must be converted to µm by multiplying it by 1000 µm /mm. For example:

0.0125 mm 1,000 µm 12.5 µm x =
ocular micrometer space mm ocular micrometer space

10.3.5.7 Follow the procedure below for each objective. Record the information as shown in the example below and keep the information available at the microscope.

Item no.	Objective power	Description	No. of ocular micrometer spaces	No. of stage micrometer mm[1]	µm/ocular micrometer space[2]
1		10X		N.A.[3]=	
2		20X		N.A.=	
3		40X		N.A.=	
4		100X		N.A.=	

[1] 100 µm /mm [2] (Stage micrometer length in mm × (1000 µm /mm)) ÷ no. ocular micrometer spaces [3] N.A. refers to numerical aperture. The numerical aperture value is engraved on the barrel of the objective.

10.3.6 Köhler illumination: This section assumes that Köhler illumination will be established for only the 100X oil DIC objective that will be used to identify internal morphological characteristics in Cryptosporidium oocysts and Giardia cysts. If more than one objective is to be used for DIC, then each time the objective is changed, Köhler illumination must be reestablished for the new objective lens. Previous sections have adjusted oculars and light sources. This section aligns and focuses the light going through the condenser underneath the stage at the specimen to be observed. If Köhler illumination is not properly established, then DIC will not work to its maximal potential. These steps need to become second nature and must be practiced regularly until they are a matter of reflex rather than a chore. The procedure must be followed each time an analyst uses the microscope and each time the objective is changed.

10.3.6.1 Place a prepared slide on the microscope stage, place oil on the slide, move the 100X oil objective into place, turn on the transmitted light, and focus the specimen image using the coarse and fine adjustment knobs.

10.3.6.2 At this point both the radiant field diaphragm in the microscope base and the aperture diaphragm in the condenser should be wide open. Now close down the radiant field diaphragm in the microscope base until the lighted field is reduced to a small opening.

10.3.6.3 Using the condenser centering screws on the front right and left of the condenser, move the small lighted portion of the field to the center of the visual field.

10.3.6.4 Now look to see whether the leaves of the iris field diaphragm are sharply defined (focused) or not. If they are not sharply defined, then they can be focused distinctly by changing the height of the condenser up and down with the condenser focusing knob while you are looking through the binoculars. Once you have accomplished the precise focusing of the radiant field diaphragm leaves, open the radiant field diaphragm until the leaves just disappear from view.

10.3.6.5 The aperture diaphragm of the condenser is now adjusted to make it compatible with the total numerical aperture of the optical system. This is done by removing an ocular, looking into the tube at the rear focal plane of the objective, and stopping down the aperture diaphragm iris leaves until they are visible just inside the rear plane of the objective.

10.3.6.6 After completing the adjustment of the aperture diaphragm in the condenser, return the ocular to its tube and proceed with the adjustments required to establish DIC

10.4 Protozoa libraries: Each laboratory is encouraged to develop libraries of photographs and drawings for identification of protozoa.

10.4.1 Take color photographs of Cryptosporidium oocysts and Giardia cysts by FA and 4',6-diamidino-2-phenylindole (DAPI) that the analysts (Section 22.2) determine are accurate (Section 15.2).

10.4.2 Similarly, take color photographs of interfering organisms and materials by FA and DAPI that the analysts believe are not Cryptosporidium oocysts or Giardia cysts. Quantify the size, shape, microscope settings, and other characteristics that can be used to differentiate oocysts and cysts from interfering debris and that will result in positive identification of DAPI positive or negative organisms.

10.5 Verification of performance: Until standard reference materials, such as National Institute of Standards and Technology standard reference materials, are available that contain a reliable number of DAPI positive or negative oocysts and cysts, this method shall rely upon the ability of the analyst for identification and enumeration of oocysts and cysts.

10.5.1 At least monthly when microscopic examinations are being performed, the laboratory shall prepare a slide containing 40 to 100 oocysts and 40 to 100 cysts. More than 50% of the oocysts and cysts must be DAPI positive.

10.5.2 Each analyst shall determine the total number of oocysts and cysts and the number that are DAPI positive or negative using the slide prepared in Section 10.5.1.

10.5.3 The total number and the number of DAPI positive or negative oocysts and cysts determined by each analyst (Section 10.5.2.) must be within ±10% of each other. If the number is not within this range, the analysts must identify the source of any variability

between analysts' examination criteria, prepare a new slide, and repeat the performance verification (Sections 10.5.1 to 10.5.2).

10.5.4 Document the date, name(s) of analyst(s), number of total, DAPI positive or negative oocysts and cysts determined by the analyst(s), whether the test was passed/failed and the results of attempts before the test was passed.

10.5.5 Only after an analyst has passed the criteria in Section 10.5.3, may oocysts and cysts in QC samples and field samples be identified and enumerated.

11.0 Oocyst and Cyst Suspension Enumeration and Spiking

11.1 This method requires routine analysis of spiked QC samples to demonstrate acceptable initial and ongoing laboratory and method performance (initial precision and recovery samples [Section 9.4], matrix spike and matrix spike duplicate samples [Section 9.5], and ongoing precision and recovery samples [Section 9.7]). The organisms used for these samples must be enumerated to calculate recoveries and precision. EPA recommends that flow cytometry be used for this enumeration, rather than manual techniques. Flow cytometer–sorted spikes generally are characterized by a relative standard deviation of ≤ 2.5%, versus greater variability for manual enumeration techniques (Reference 20.8). Guidance on preparing spiking suspensions using a flow cytometer is provided in Section 11.2. Manual enumeration procedures are provided in Section 11.3. The procedure for spiking bulk samples in the laboratory is provided in Section 11.4.

11.2 Flow cytometry enumeration guidelines. Although it is unlikely that many laboratories performing Method 1623 will have direct access to a flow cytometer for preparing spiking suspensions, flow-sorted suspensions are available from commercial vendors and other sources (Section 7.10.1). The information provided in Sections 11.2.1 through 11.2.4 is simply meant as a guideline for preparing spiking suspensions using a flow cytometer. Laboratories performing flow cytometry must develop and implement detailed standardized protocols for calibration and operation of the flow cytometer.

11.2.1 Spiking suspensions should be prepared using unstained organisms that have not been heat-fixed or formalin-fixed.

11.2.2 Spiking suspensions should be prepared using Cryptosporidium parvum oocysts <3 months old, and Giardia intestinalis cysts <2 weeks old.

11.2.3 Initial calibration. Immediately before sorting spiking suspensions, an initial calibration of the flow cytometer should be performed by conducting 10 sequential sorts directly onto membranes or well slides. The oocyst and cyst levels used for the initial calibration should be the same as the levels used for the spiking suspensions. Each initial calibration sample should be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The relative standard deviation (RSD) of the 10 counts should be ≤ 2.5%. If the RSD is > 2.5%, the laboratory should perform the initial calibration again, until the RSD of the 10 counts is ≤ 2.5%. In addition to counting the organisms, the laboratory also should evaluate the quality of the organisms using DAPI and DIC to confirm that the organisms are in good condition.

11.2.4 Ongoing calibration. When sorting the spiking suspensions for use in QC samples, the laboratory should perform ongoing calibration samples at a 10% frequency, at a minimum. The laboratory should sort the first run and every eleventh sample directly onto a membrane or well slide. Each ongoing calibration sample should be stained and manually counted microscopically and the manual counts used to verify the accuracy of the system. The mean of the ongoing calibration counts also should be used as the estimated spike dose, if the relative standard deviation (RSD) of the ongoing calibration counts is ≤ 2.5%. If the RSD is > 2.5%, the laboratory should discard the batch.

11.2.5 Method blanks. Depending on the operation of the flow cytometer, method blanks should be prepared and examined at the same frequency as the ongoing calibration samples (Section 11.2.4).

11.2.6 Holding time criteria. Flow-cytometer-sorted spiking suspensions (Sections 7.10.1 and 11.2) used for spiked quality control (QC) samples (Section 9) must be used within the expiration date noted on the suspension. Laboratories

should use flow-cytometer-sorted spiking suspensions containing live organisms within two weeks of preparation at the flow cytometry laboratory.

11.3 Manual enumeration procedures. Two sets of manual enumerations are required per organism before purified Cryptosporidium oocyst and Giardia cyst stock suspensions (Sections 7.9.2.1 and 7.9.2.2) received from suppliers can be used to spike samples in the laboratory. First, the stock suspension must be diluted and enumerated (Section 11.3.3) to yield a suspension at the appropriate oocyst or cyst concentration for spiking (spiking suspension). Then, 10 aliquots of spiking suspension must be enumerated to calculate a mean spike dose. Spiking suspensions can be enumerated using hemacytometer chamber counting (Section 11.3.4), well slide counting (Section 11.3.5), or membrane filter counting (Section 11.3.6).

11.3.1 Precision criteria. The relative standard deviation (RSD) of the calculated mean spike dose for manually enumerated spiking suspensions must be ≤16% for *Cryptosporidium* and ≤19% for *Giardia* before proceeding (these criteria are based on the pooled RSDs of 105 manual *Cryptosporidium* enumerations and 104 manual *Giardia* enumerations submitted by 20 different laboratories under the EPA Protozoa Performance Evaluation Program).

11.3.2 Holding time criteria. Manually enumerated spiking suspensions must be used within 24 hours of enumeration of the spiking suspension if the hemacytometer chamber technique is used (Section 11.3.4); or within 24 hours of application of the spiking suspension or membrane filter to the slides if the well slide or membrane filter enumeration technique is used (Sections 11.3.5 and 11.3.6).

11.3.3 Enumerating and diluting stock suspensions

11.3.3.1 Purified, concentrated stock suspensions (Sections 7.10.2.1 and 7.10.2.2) must be diluted and enumerated before the diluted suspensions are used to spike samples in the laboratory. Stock suspensions should be diluted with reagent water/Tween-20, 0.01% (Section 7.10.2.3), to a concentration of 20 to 50 organisms per large hemacytometer square before proceeding to Section 11.3.3.2.

11.3.3.2 Apply a clean hemacytometer coverslip (Section 6.4.5) to the hemacytometer and load the hemacytometer chamber with 10 µL of vortexed suspension per chamber. If this operation has been properly executed, the liquid should amply fill the entire chamber without bubbles or overflowing into the surrounding moats. Repeat this step with a clean, dry hemacytometer and coverslip if loading has been incorrectly performed. See Section 11.3.3.13, below, for the hemacytometer cleaning procedure.

11.3.3.3 Place the hemacytometer on the microscope stage and allow the oocysts or cysts to settle for 2 minutes Do not attempt to adjust the coverslip, apply clips, or in any way disturb the chamber after it has been filled.

11.3.3.4 Use 200X magnification.

11.3.3.5 Move the chamber so the ruled area is centered underneath it.

11.3.3.6 Move the objective close to the coverslip while watching it from the side of the microscope, rather than through the microscope.

11.3.3.7 Focus up from the coverslip until the hemacytometer ruling appears.

11.3.3.8 At each of the four corners of the chamber is a 1-square-mm area divided into 16 squares in which organisms are to be counted (Figure 1). Beginning with the top row of four squares, count with a hand-tally counter in the directions indicated in Figure 2. Avoid counting organisms twice by counting only those touching the top and left boundary lines. Count each square millimeter in this fashion.

11.3.3.9 Use the following formula to determine the number of organisms per mL of suspension:

11.3.3.10 Record the result on a hemacytometer data sheet.

11.3.3.11 A total of six different hemacytometer chambers must be loaded, counted, and averaged for each suspension to achieve optimal counting accuracy.

11.3.3.12 Based on the hemacytometer counts, the stock suspension should be diluted to a final concentration of between 8000 and 12,000 organisms per mL (80 to 120 organisms per 10

$_{\mu L}$); however, ranges as great as 5000 to 15,000 organisms per mL (50 to 150 organisms per 10 µL) can be used.

NOTE: If the diluted stock suspensions (the spiking suspensions) will be enumerated using hemacytometer chamber counts (Section 11.3.4) or membrane filter counts (Section 11.3.6), then the stock suspensions should be diluted with 0.01% Tween-20. If the spiking suspensions will be enumerated using well slide counts (Section 11.3.3), then the stock suspensions should be diluted in reagent water.

To calculate the volume (in µL) of stock suspension required per mL of reagent water (or reagent water/Tween-20, 0.01%), use the following formula:

required number of organisms x 1000 µL volume of stock suspension (µL) required = number of organisms/mL of Stock suspension

If the volume is less than 10 µL , an additional dilution of the stock suspension is recommended before proceeding.

To calculate the dilution factor needed to achieve the required number of organisms per 10 µL , use the following formula:
Total volume (µL) number of organisms required x 10 µL predicted number of organisms per 10 µL (80 to 120)

To calculate the volume of reagent water (or reagent water/Tween-20, 0.01%) needed, use the following formula:
reagent water volume (µL) = total volume (µL) -stock suspension volume required (µL)
11.3.3.13 After each use, the hemacytometer and coverslip must be cleaned immediately to prevent the organisms and debris from drying on it. Since this apparatus is precisely machined, abrasives cannot be used to clean it, as they will disturb the flooding and volume relationships.
11.3.3.13.1 Rinse the hemacytometer and cover glass first with tap water, then 70% ethanol, and finally with acetone.
11.3.3.13.2 Dry and polish the hemacytometer chamber and cover glass with lens paper. Store it in a secure place.

11.3.3.14 Several factors are known to introduce errors into hemacytometer counts, including:

- Inadequate mixing of suspension before flooding the chamber.
- Irregular filling of the chamber, trapped air bubbles, dust, or oil on the chamber or coverslip.
- Total number of organisms counted is too low to provide statistical confidence in the result
- Error in recording tally.
- Calculation error; failure to consider dilution factor, or area counted.
- Inadequate cleaning and removal of organisms from the previous count.
- Allowing filled chamber to sit too long, so that the chamber suspension dries and concentrates.

11.3.4 Enumerating spiking suspensions using a hemacytometer chamber
NOTE: Spiking suspensions enumerated using a hemacytometer chamber must be used within 24 hours of enumeration.
11.3.4.1 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.
11.3.4.2 To an appropriate-size beaker containing a stir bar, add enough spiking suspension to perform all spike testing and the enumeration as described. The liquid volume and beaker relationship should be such that a spinning stir bar does not splash the sides of the beaker, the stir bar has unimpeded rotation, and there is enough room to draw sample from the beaker with a 10-µL micropipette without touching the stir bar. Cover the beaker with a watch glass or Petri dish to prevent evaporation between sample withdrawals.
11.3.4.3 Allow the beaker contents to stir for a minimum of 30 minutes before beginning enumeration.
11.3.4.4 While the stir bar is still spinning, remove a 10-µL aliquot and carefully load one side of the hemacytometer. Count all organisms on the platform, at 200X magnification using phase-

contrast or darkfield microscopy. The count must include the entire area under the hemacytometer, not just the four outer 1-mm^2 squares. Repeat this procedure nine times. This step allows confirmation of the number of organisms per 10 µL (Section 11.3.3.12). Based on the 10 counts, calculate the mean, standard deviation, and RSD of the counts. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts. Refer to Section 11.3.3.14 for factors that may introduce errors.

Enumerating spiking suspensions using well slides

NOTE: Spiking suspensions enumerated using well slides must be used within 24 hours of application of the spiking suspension to the slides.

11.3.5.1 Remove well slides from cold storage and lay the slides on a flat surface for 15 minutes to allow them to warm to room temperature.

11.3.5.2 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.5.3 Remove a 10-µL aliquot from the spiking suspension and apply it to the center of a well.

11.3.5.4 Before removing subsequent aliquots, cap the tube and gently invert it three times to ensure that the oocysts or cysts are in suspension.

11.3.5.5 Ten wells must be prepared and counted, and the counts averaged, to sufficiently enumerate the spike dose. Air-dry the well slides. Because temperature and humidity varies from laboratory to laboratory, no minimum time is specified. However, the laboratory must take care to ensure that the sample has dried completely before staining to prevent losses during the rinse steps. A slide warmer set at 35 ^{o}C to 42 ^{o}C also can be used.

11.3.5.6 Positive and negative controls must be prepared.

11.3.5.6.1 For the positive control, pipette 10 µL of positive antigen or 200 to 400 intact oocysts or cysts to the center of a well and distribute evenly over the well area.

11.3.5.6.2 For the negative control, pipette 50 µL of PBS onto the center of a well and spread it over the well area with a pipette tip.

11.3.5.6.3 Air-dry the control slides.

11.3.5.7 Apply 50-µL of absolute methanol to each well containing the dried sample and allow to air-dry for 3 to 5 minutes.

11.3.5.8 Follow the manufacturer's instructions (Section 7.6) in applying the stain to the slide.

11.3.5.9 Place the slides in a humid chamber in the dark and incubate at room temperature for approximately 30 minutes. The humid chamber consists of a tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

11.3.5.10 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess detection reagent from below the well using a clean Pasteur pipette or absorb with a paper towel or other absorbent material. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

11.3.5.11 Add mounting medium (Section 7.8) to each well.

11.3.5.12 Apply a cover slip. Use a tissue to remove excess mounting fluid from the edges of the coverslip. Seal the edges of the coverslip onto the slide using clear nail polish.

11.3.5.13 Record the date and time that staining was completed. If slides will not be read immediately, store in a humid chamber in the dark at 0 ^{o}C to 8 ^{o}C until ready for examination.

11.3.5.14 After examination of the 10 wells, calculate the mean, standard deviation, and RSD of the 10 replicates. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or

dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts.

11.3.6 Enumeration of spiking suspensions using membrane filters

NOTE: Spiking suspensions enumerated using membrane filters must be used within 24 hours of application of the filters to the slides.

11.3.6.1 Pre-coat the glass funnels with Sigmacote® by placing the funnel in a large Petri dish and applying 5-mL of Sigmacoat® to the funnel opening using a pipette and allowing it to run down the inside of the funnel. Repeat for all funnels to be used. The pooled Sigmacoat® may be returned to the bottle for re-use. Place the funnels at 35 ^{o}C or 41 ^{o}C for approximately 5 minutes to dry.

11.3.6.2 Place foil around the bottoms of the 100 × 15 mm Petri dishes.

11.3.6.3 Filter-sterilize (Section 6.19) approximately 10 mL of PBS pH 7.2 (Section 7. 9. 4). Dilute detection reagent (Section 7.7) as per manufacturer's instructions using sterile PBS. Multiply the anticipated number of filters to be stained by 100 mL to calculate total volume of stain required. Divide the total volume required by 5 to obtain the microliters of antibody necessary. Subtract the volume of antibody from the total stain volume to obtain the required microliters of sterile PBS to add to the antibody.

11.3.6.4 Label the tops of foil-covered, 60 × 15 mm Petri dishes for 10 spiking suspensions plus positive and negative staining controls and multiple filter blanks controls (one negative control, plus a blank after every five sample filters to control for carry-over). Create a humid chamber by laying damp paper towels on the bottom of a stain tray (the inverted foil-lined Petri dishes will protect filters from light and prevent evaporation during incubation).

11.3.6.5 Place a decontaminated and cleaned filter holder base (Section 6.4.8.1) into each of the three ports of the vacuum manifold (Section 6.4.8.2).

11.3.6.6 Pour approximately 10 mL of 0.01% Tween 20 into a 60 × 15 mm Petri dish.

11.3.6.7 Using forceps, moisten a 1.2-µm cellulose-acetate support membrane (Section 6.4.8.3) in the 0.01% Tween 20 and place it on the fritted glass support of one of the filter bases. Moisten a polycarbonate filter (Section 6.4.8.4) the same way and position it on top of the cellulose-acetate support membrane. Carefully clamp the glass funnel to the loaded filter support. Repeat for the other two filters.

11.3.6.8 Add 5 mL of 0.01% Tween 20 to each of the three filtration units and allow to stand.

11.3.6.9 Vortex the tube containing the spiking suspension (diluted stock suspension; Section 11.3.3) for a minimum of 2 minutes. Gently invert the tube three times.

11.3.6.10 Using a micropipettor, sequentially remove two, 10-µL aliquots from the spiking suspension and pipet into the 5 mL of 0.01% Tween 20 standing in the unit. Rinse the pipet tip twice after each addition. Apply 10 µL of 0.01% Tween 20 to the third unit to serve as the negative control. Apply vacuum at 2" Hg and allow liquid to drain to miniscus, then close off vacuum. Pipet 10 mL of reagent water into each funnel and drain to miniscus, closing off the vacuum. Repeat the rinse and drain all fluid, close off the vacuum.

11.3.6.11 Pipet 100 mL of diluted antibody to the center of the bottom of a 60 × 15 mm Petri dish for each sample.

11.3.6.12 Unclamp the top funnel and transfer each cellulose acetate support membrane/ polycarbonate filter combination onto the drop of stain using forceps (apply each membrane/filter combination to a different Petri dish containing stain). Roll the filter into the drop to exclude air. Place the small Petri dish containing the filter onto the damp towel and cover with the corresponding labeled foil-covered top. Incubate for approximately 45 minutes at room temperature.

11.3.6.13 Reclamp the top funnels, apply vacuum and rinse each three times, each time with 20 mL of reagent water.

11.3.6.14 Repeat Sections 11.3.6.4 through 11.3.6.10 for the next three samples (if that the diluted spiking suspension has sat less than 15 minutes, reduce the suspension vortex time to 60 seconds). Ten, 10-µL spiking suspension aliquots must be prepared and counted, and the counts averaged, to sufficiently enumerate the spike dose. Include a filter blank sample at a

frequency of every five samples; rotate the position of filter blank to eventually include all three filter placements.
11.3.6.15 Repeat Sections 11.3.6.4 through 11.3.6.10 until the 10-µL spiking suspensions have been filtered. The last batch should include a 10-µL 0.01 Tween 20 blank control and 20 µL of positive control antigen as a positive staining control.
11.3.6.16 Label slides. After incubation is complete, for each sample, transfer the cellulose acetate filter support and polycarbonate filter from drop of stain and place on fritted glass support. Cycle vacuum on and off briefly to remove excess fluid. Peel the top polycarbonate filter off the supporting filter and place on labeled slide. Discard cellulose acetate filter support. Mount and apply coverslips to the filters immediately to avoid drying.
11.3.6.17 To each slide, add 20 µL of mounting medium (Section 7.8).
11.3.6.18 Apply a coverslip. Seal the edges of the coverslip onto the slide using clear nail polish. (Sealing may be delayed until cover slips are applied to all slides.)
11.3.6.19 Record the date and time that staining was completed. If slides will not be read immediately, store sealed slides in a closed container in the dark at 0 ^{o}C to 8 ^{o}C until ready for examination.
11.3.6.20 After examination of the 10 slides, calculate the mean, standard deviation, and RSD of the 10 replicates. Record the counts and the calculations on a spiking suspension enumeration form. The relative standard deviation (RSD) of the calculated mean spike dose must be ≤16% for Cryptosporidium and ≤19% for Giardia before proceeding. If the RSD is unacceptable, or the mean number is outside the expected range, add additional oocysts from stock suspension or dilute the contents of the beaker appropriately with reagent water. Repeat the process to confirm counts.
11.3.6.21 If oocysts or cysts are detected on the filter blanks, modify the rinse procedure to ensure that no carryover occurs and repeat enumeration.
11.4 Procedure for spiking samples in the laboratory with enumerated spiking suspensions.
11.4.1 Arrange a bottom-dispensing container to feed the filter.
11.4.2 For initial precision and recovery (Section 9.4) and ongoing precision and recovery (Section 9.7) samples, fill the container with a volume of reagent water equal to the volume of the field samples analyzed in the analytical batch. For matrix spike samples (Section 9.5), fill the container with the field sample to be spiked. Continuously mix the sample (using a stir bar and stir plate for smaller-volume samples and alternate means for larger-volume samples).
11.4.3 Vortex the spiking suspension(s) (Section 11.2 or Section 11.3) for a minimum of 2 minutes.

11.4.3.1 For flow cytometer–enumerated suspensions (where the entire volume of a spiking suspension tube will be used):

11.4.3.1.1 Add 500 µL of the diluted antifoam to the tube containing the spiking suspension and vortex for 2 minutes.
11.4.3.1.2 Pour the suspension into the sample container.
11.4.3.1.3 Add 20 mL of reagent water to the empty tube, cap, vortex 10 seconds to rinse, and add the rinsate to the carboy.
11.4.3.1.4 Repeat this rinse using another 20 mL of reagent water.
11.4.3.1.5 Record the estimated number of organisms spiked, the date and time the sample was spiked, and the sample volume spiked on a bench sheet.
11.4.3.1.6 Proceed to Section 11.4.4.
11.4.3.2 For manually enumerated spiking suspensions:

11.4.3.2.1 Rinse a pipette tip with 0.01% Tween-20 once, then rinse with the well-mixed spiking suspension a minimum of five times before pulling an aliquot to be used to spike the container.

11.4.3.2.2 Add the spiking suspension(s) to the carboy, delivering the aliquot below the surface of the water.

11.4.3.2.3 Record the estimated number of organisms spiked, the date and time the sample was spiked, and the sample volume spiked on a bench sheet. Proceed to Section 11.4.4

11.4.4 Allow the spiking suspensions to mix for approximately 1 minute in the container.

11.4.5 Turn on the pump and allow the flow rate to stabilize. Set flow at the rate designated for the filter being used. As the carboy is depleted, check the flow rate and adjust if necessary.
11.4.6 When the water level approaches the discharge port of the carboy, tilt the container so that it is completely emptied. At that time, turn off the pump and add sufficient reagent water to the container to rinse. Swirl the contents to rinse down the sides.
11.4.7 Turn on the pump. Allow all of the water to flow through the filter and turn off the pump.

12.0 Sample Filtration and Elution
12.1 A water sample is filtered according to the procedures in Section 12.2. Alternate procedures may be used if the laboratory first demonstrates that the alternate procedure provides equivalent or superior performance per Section 9.1.2.
NOTE: Sample elution must be initiated within 96 hours of sample collection (if shipped to the laboratory as a bulk sample) or filtration (if filtered in the field).

12.2 Capsule filtration (adapted from Reference 20.9). This procedure was validated using 10-L sample volumes. Alternate sample volumes may be used, provided the laboratory demonstrates acceptable performance on initial and ongoing spiked reagent water and source water samples (Section 9.1.2).
NOTE: The filtration procedures specified in Section 12.2.1 - 12.2.5.3 are specific to laboratory filtration of a bulk sample, and reflect the procedures used during the interlaboratory validation of this method (Reference 20.10). These procedures may require modification if samples will be filtered in the field.

12.2.1 Flow rate adjustment

12.2.1.1 Connect the sampling system, minus the capsule, to a carboy filled with reagent water (Figure 3).
12.2.1.2 Turn on the pump and adjust the flow rate to 2.0 L/min.
12.2.1.3 Allow 2 to 10 L of reagent water to flush the system. Adjust the pump speed as required during this period. Turn off the pump when the flow rate has been adjusted.

12.2.2 Install the capsule filter in the line, securing the inlet and outlet ends with the appropriate clamps/fittings.
12.2.3 Record the sample number, sample turbidity (if not provided with the field sample), sample type, and sample filtration start date and time on a bench sheet.

12.2.4 Filtration
12.2.4.1 Connect the sampling system to the field carboy of sample water, or transfer the sample water to the laboratory carboy used in Section
12.2.1.1. If the sample will be filtered from a field carboy, a spigot (Section 6.2.1) can be used with the carboy to facilitate sample filtration.
NOTE: If the bulk field sample is transferred to a laboratory carboy, the laboratory carboy must be cleaned and disinfected before it is used with another field sample.
12.2.4.2 Place the drain end of the sampling system tubing into an empty graduated container with a capacity of 10 to 15 L, calibrated at 9.0, 9.5, 10.0, 10.5, and 11.0 L (Section 6.18). This container will be used to determine the sample volume filtered. Alternately, connect a flow meter (Section 6.3.4) downstream of the filter, and record the initial meter reading.
12.2.4.3 Allow the carboy discharge tube and capsule to fill with sample water. Vent residual air using the bleed valve/vent port, gently shaking or tapping the capsule, if necessary. Turn on the pump to start water flowing through the filter. Verify that the flow rate is 2 L/min.
12.2.4.4 After all of the sample has passed through the filter, turn off the pump. Allow the pressure to decrease until flow stops. (If the sample was filtered in the field, and excess sample remains in the filter upon receipt in the laboratory, pull the remaining sample volume through the filter before eluting the filter [Section 12.2.6].)

12.2.5 Disassembly

12.2.5.1 Disconnect the inlet end of the capsule filter assembly while maintaining the level of the inlet fitting above the level of the outlet fitting to prevent backwashing and the loss of oocysts and cysts from the filter. Restart the pump and allow as much water to drain as possible. Turn off the pump.

12.2.5.2 Based on the water level in the graduated container or meter reading, record the volume filtered on the bench sheet to the nearest quarter liter. Discard the contents of the graduated container.

12.2.5.3 Loosen the outlet fitting, then cap the inlet and outlet fittings.

12.2.6 Elution

NOTE: The laboratory must complete the elution, concentration, and purification (Sections 12.2.6 through 13.3.3.11) in one work day. It is critical that these steps be completed in one work day to minimize the time that any target organisms present in the sample sit in eluate or concentrated matrix. This process ends with the application of the purified sample on the slide for drying.

12.2.6.1 Setup

12.2.6.1.1 Assemble the laboratory shaker with the clamps aligned vertically so that the filters will be aligned horizontally. Extend the clamp arms to their maximum distance from the horizontal shaker rods to maximize the shaking action.

12.2.6.1.2 Prepare sufficient elution buffer so that all samples to be eluted that day can be eluted with the same batch of buffer. Elution may require up to 275 mL of buffer per sample.

12.2.6.1.3 Designate at least one 250-mL conical centrifuge tube for each sample and label with the sample number.

12.2.6.2 Elution

12.2.6.2.1 Record the elution date and time on the bench sheet. Using a ring stand or other means, clamp each capsule in a vertical position with the inlet end up. Remove the inlet cap and allow the liquid level to stabilize.

12.2.6.2.2 Pour elution buffer through the inlet fitting. Sufficient elution buffer must be added to cover the pleated white membrane with buffer solution. Replace the inlet cap and clamp the cap in place.

12.2.6.2.3 Securely clamp the capsule in one of the clamps on the laboratory shaker with the bleed valve positioned at the top on a vertical axis (in the 12 o'clock position). Turn on the shaker and set the speed to maximum (approximately 900 rpm). Agitate the capsule for approximately 5 minutes. Time the agitation using a lab timer, rather than the timer on the shaker to ensure accurate time measurement.

12.2.6.2.4 Remove the filter from the shaker, remove the inlet cap, and pour the contents of the capsule into the 250-mL conical centrifuge tube.

12.2.6.2.5 Clamp the capsule vertically with the inlet end up and add sufficient volume of elution buffer through the inlet fitting to cover the pleated membrane. Replace the inlet cap.

12.2.6.2.6 Return the capsule to the shaker with the bleed valve positioned at the 4 o'clock position. Turn on the shaker and agitate the capsule for approximately 5 minutes.

12.2.6.2.7 Remove the filter from the shaker, but leave the elution buffer in the capsule. Re-clamp the capsule to the shaker at the 8 o'clock position. Turn on the shaker and agitate the capsule for a final 5 minutes.

12.2.6.2.8 Remove the filter from the shaker and pour the contents into the 250-mL centrifuge tube. Rinse down the inside of the capsule filter walls with reagent water or elution buffer using a squirt bottle inserted in the inlet end of the capsule. Invert the capsule filter over the centrifuge tube and ensure that as much of the eluate as possible has been transferred.

12.2.7 Proceed to Section 13.0 for concentration and separation (purification).

13.0 Sample Concentration and Separation (Purification)

13.1 During concentration and separation, the filter eluate is concentrated through centrifugation, and the oocysts and cysts in the sample are separated from other particulates through immunomagnetic separation (IMS). Alternate procedures and

products may be used if the laboratory first demonstrates equivalent or superior performance as per Section 9.1.2.

13.2 Adjustment of pellet volume

13.2.1 Centrifuge the 250-mL centrifuge tube containing the capsule filter eluate at 1500 × G for 15 minutes. Allow the centrifuge to coast to a stop—do not use the brake. Record the pellet volume (volume of solids) on the bench sheet.

NOTE: Recoveries may be improved if centrifugation force is increased to 2000 × G. However, do not use this higher force if the sample contains sand or other gritty material that may degrade the condition of any oocysts and/or cysts in the sample.

13.2.2 Using a Pasteur pipette, carefully aspirate the supernatant to 5 mL above the pellet. Extra care must be taken to avoid aspirating oocysts and cysts during this step, particularly if the sample is reagent water (e.g. initial or ongoing precision and recovery sample).

13.2.3 If the packed pellet volume is ≤ 0.5 mL, vortex the tube vigorously until pellet is completely resuspended. Swirl the centrifuge tube gently to reduce any foaming after vortexing. Record the resuspended pellet volume on the bench sheet. Proceed to Section 13.3.

NOTE: Extra care must be taken with samples containing sand or other gritty material when vortexing to ensure that the condition of any oocysts and/or cysts in the sample is not compromised.

13.2.4 If the packed pellet volume is > 0.5 mL, the concentrate needs to be separated into multiple subsamples (a subsample is equivalent to no greater than 0.5 mL of packed pellet material, the recommended maximum amount of particulate material to process through the subsequent purification and examination steps in the method). Use the following formula to determine the total volume required in the centrifuge tube before separating the concentrate into two or more subsamples:

$$\text{total volume (mL) required} = \frac{\text{pellet volume}}{0.5\ \text{mL}} \times 5\ \text{mL}$$

(For example, if the packed pellet volume is 1.2 mL, the total volume required is 12 mL.) Add reagent water to the centrifuge tube to bring the total volume to the level calculated above. Vortex the tube vigorously for 10 to 15 seconds to completely resuspend the pellet. Record the resuspended pellet volume on the bench sheet.

> **NOTE**: Extra care must be taken with samples containing sand or other gritty material when vortexing to ensure that the condition of any oocysts in the sample is not compromised.

13.2.4.1 Analysis of entire sample. If analysis of the entire sample is required, determine the number of subsamples to be processed independently through the remainder of the method:

13.2.4.1.1 Calculate number of subsamples: Divide the total volume in the centrifuge tube by 5 mL and round up to the nearest integer (for example, if the resuspended volume in Section 13.2.4 is 12 mL, then the number of subsamples would be 12 mL / 5 mL = 2.4, rounded = 3 subsamples).

13.2.4.1.2 Determine volume of resuspended concentrate per subsample. Divide the total volume in the centrifuge tube by the calculated number of subsamples (for 13.2.4.1.3 example, if the resuspended volume in Section 13.2.4 is 12 mL, then the volume to use for each subsample = 12 mL / 3 subsamples = 4 mL).

Process sub-samples through IMS. Proceed to Section 13.3, and transfer aliquots of the resuspended concentrate equivalent to the volume in the previous step to multiple, flat-sided sample tubes in Section 13.3.2.1. Process the sample as multiple, independent subsamples from Section 13.3 onward, including the preparation and examination of separate slides for each aliquot. Record the volume of resuspended concentrate transferred to IMS on the bench sheet

(this will be equal to the volume recorded in Section 13.2.4). Also record the number of subsamples processed independently through the method on the bench sheet.

13.2.4.2 Analysis of partial sample. If not all of the concentrate will be examined, proceed to Section 13.3, and transfer one or more 5-mL aliquots of the resuspended concentrate to one or more flat-sided sample tubes in Section 13.3.2.1. Record the volume of resuspended concentrate transferred to IMS on the bench sheet. To determine the volume analyzed, calculate the percent of the concentrate examined using the following formula:

$$\text{percent examined} = \frac{\text{total volume of resuspended concentrate transferred to IMS}}{\text{total volume of resuspended concentrate in Section 13.2.4}} \times 100\%$$

Then multiply the volume filtered (Section 12.2.5.2) by this percentage to determine the volume analyzed.

13.3 IMS procedure (adapted from Reference 20.11)

NOTE: The IMS procedure should be performed on a bench top with all materials at room temperature, ranging from 15 ^{o}C to 25 ^{o}C.

13.3.1 Preparation and addition of reagents

13.3.1.1 Prepare a 1X dilution of SL-buffer-A from the 10X SL-buffer-A (clear, colorless solution) supplied. Use reagent water (demineralized; Section 7.3) as the diluent. For every 1 mL of 1X SL-buffer-A required, take 100 µL of 10X SL-buffer-A and make up to 1 mL with the diluent water. A volume of 1.5 mL of 1X SL-buffer-A will be required per sample or subsample on which the Dynal IMS procedure is performed.

13.3.1.2 For each sample or subsample (Section 13.2) to be processed through IMS, add 1 mL of the 10X SL-buffer-A (supplied—not the diluted 1X SL-buffer-A) to a flat-sided tube (Section 6.5.4).

13.3.1.3 For each subsample, add 1 mL of the 10X SL-buffer-B (supplied— magenta solution) to the flat-sided tube containing the 10X SL-buffer-A.

13.3.2 Oocyst and cyst capture

13.3.2.1 Use a graduated, 10-mL pipette that has been pre-rinsed with elution buffer to transfer the water sample concentrate from Section 13.2 to the flat-sided tube(s) containing the SL-buffer. If all of the concentrate is used, rinse the centrifuge tube twice with reagent water and add the rinsate to the flat-sided tube containing the concentrate (or to the tube containing the first subsample, if multiple subsamples will be processed). Each of the two rinses should be half the volume needed to bring the total volume in the flat-sided sample tube to 10 mL. (For example, if 5 mL was transferred after resuspension of the pellet, the centrifuge tube would be rinsed twice with 2.5 mL of reagent water to bring the total volume in the flat-sided tube to 10 mL.) Visually inspect the centrifuge tube after completing the transfer to ensure that no concentrate remains. If multiple subsamples will be processed, bring the volume in the remaining flat-sided tubes to 10 mL with reagent water. Label the flat-sided tube(s) with the sample number (and subsample letters).

13.3.2.2 Vortex the Dynabeads®Crypto-Combo vial from the IMS kit for approximately 10 seconds to suspend the beads. Ensure that the beads are fully resuspended by inverting the sample tube and making sure that there is no residual pellet at the bottom.

13.3.2.3 Add 100 µL of the resuspended Dynabeads®Crypto-Combo (Section 13.3.2.2) to the sample tube(s) containing the water sample concentrate and SL-buffer.

13.3.2.4 Vortex the Dynabeads®Giardia-Combo vial from the IMS kit for approximately 10 seconds to suspend the beads. Ensure that the beads are fully resuspended by inverting the tube and making sure that there is no residual pellet at the bottom.

13.3.2.5 Add 100 µL of the resuspended Dynabeads®Giardia-Combo (Section 13.3.2.4) to the sample tube(s) containing the water sample concentrate, Dynabeads®Crypto-Combo, and SL-buffer.

13.3.2.6 Affix the sample tube(s) to a rotating mixer and rotate at approximately 18 rpm for 1 hour at room temperature.

13.3.2.7 After rotating for 1 hour, remove each sample tube from the mixer and place the tube in the magnetic particle concentrator (MPC-1) with flat side of the tube toward the magnet.

13.3.2.8 Without removing the sample tube from the MPC-1, place the magnet side of the MPC-1 downwards, so the tube is horizontal and the flat side of the tube is facing down.

13.3.2.9 Gently rock the sample tube by hand end-to-end through approximately 90°, tilting the cap-end and base-end of the tube up and down in turn. Continue the tilting action for 2 minutes with approximately one tilt per second.

13.3.2.10 Ensure that the tilting action is continued throughout this period to prevent binding of low-mass, magnetic or magnetizable material. If the sample in the MPC-1 is allowed to stand motionless for more than 10 seconds, repeat Section 13.3.2.9 before continuing to Section 13.3.2.11.

13.3.2.11 Return the MPC-1 to the upright position, sample tube vertical, with cap at top. Immediately remove the cap and, keeping the flat side of the tube on top, pour off all of the supernatant from the tube held in the MPC-1 into a suitable container. Do not shake the tube and do not remove the tube from MPC-1 during this step.

13.3.2.12 Remove the sample tube from the MPC-1 and resuspend the sample in 1-mL 1X SL-buffer-A (prepared from 10X SL-buffer-A stock—supplied). Mix very gently to resuspend all material in the tube. Do not vortex.

13.3.2.13 Quantitatively transfer (transfer followed by two rinses) all the liquid from the sample tube to a labeled, 1.5-mL microcentrifuge tube. Use 1 mL of 1X SL-buffer-A to perform the first rinse and 0.5 mL of reagent water for the second rinse. Liberally rinse down the sides of the Leighton tube before transferring. Allow the flat-sided sample tube to sit for a minimum of 1 minute after transfer of the second rinse volume, then use a pipette to collect any residual volume that drips down to the bottom of the tube to ensure that as much sample volume is recovered as possible. Ensure that all of the liquid and beads are transferred.

13.3.2.14 Place the microcentrifuge tube into the second magnetic particle concentrator (MPC-M), with its magnetic strip in place.

13.3.2.15 Without removing the microcentrifuge tube from MPC-M, gently rock/roll the tube through 180° by hand. Continue for approximately 1 minute with approximately one 180° roll/rock per second. At the end of this step, the beads should produce a distinct brown dot at the back of the tube.

13.3.2.16 Immediately aspirate the supernatant from the tube and cap held in the MPC-M. If more than one sample is being processed, conduct three 90° rock/roll actions before removing the supernatant from each tube. Take care not to disturb the material attached to the wall of the tube adjacent to the magnet. Do not shake the tube. Do not remove the tube from MPC-M while conducting these steps.

13.3.3 Dissociation of beads/oocyst/cyst complex

NOTE: Two acid dissociations are required.

13.3.3.1 Remove the magnetic strip from the MPC-M.

13.3.3.2 Add 50 µL of 0.1 N HCl, then vortex at the highest setting for approximately 50 seconds.

NOTE: The laboratory should use 0.1-N standards purchased directly from a vendor, rather than adjusting the normality in-house.

13.3.3.3 Place the tube in the MPC-M without the magnetic strip in place and allow to stand in a vertical position for at least 10 minutes at room temperature.

13.3.3.4 Vortex vigorously for approximately 30 seconds.

13.3.3.5 Ensure that all of the sample is at the base of the tube. Place the microcentrifuge tube in the MPC-M.

13.3.3.6 Replace magnetic strip in MPC-M and allow the tube to stand undisturbed for a minimum of 10 seconds.
13.3.3.7 Prepare a well slide for sample screening and label the slide.
13.3.3.8 Add 5 µL of 1.0 N NaOH to the sample wells of two well slides (add 10 µL to the sample well of one well slide if the volume from the two required dissociations will be added to the same slide).

NOTE: The laboratory should use 1.0-N standards purchased directly from a vendor rather than adjusting the normality in-house.
13.3.3.9 Without removing the microcentrifuge tube from the MPC-M, transfer all of the sample from the microcentrifuge tube in the MPC-M to the sample well with the NaOH. Do not disturb the beads at the back wall of the tube. Ensure that all of the fluid is transferred.
13.3.3.10 Do not discard the beads or microcentrifuge tube after transferring the volume from the first acid dissociation to the well slide. Perform the steps in Sections 13.3.3.1 through 13.3.3.9 a second time. The volume from the second dissociation can be added to the slide containing the volume from the first dissociation, or can be applied to a second slide.

NOTE: If one slide is used, exert extra care when using Dynal Spot-On slides to ensure that the sample stays within the smaller-diameter wells on these slides.

13.3.3.11 Record the date and time the purified sample was applied to the slide(s).
13.3.3.12 Air-dry the sample on the well slide(s). Because temperature and humidity varies from laboratory to laboratory, no minimum time is specified. However, the laboratory must take care to ensure that the sample has dried completely before staining to prevent losses during the rinse steps. A slide warmer set at 35 ^{o}C to 42 ^{o}C also can be used.

14.0 Sample Staining

NOTE: The sample must be stained within 72 hours of application of the purified sample to the slide.

14.1 Prepare positive and negative controls.
14.1.1 For the positive control, pipette 10 µL of positive antigen or 200 to 400 intact oocysts and 200 to 400 cysts to the center of a well.
14.1.2 For the negative control, pipette 50 µL of 150 mM PBS (Section 7.6.4) into the center of a well and spread it over the well area with a pipette tip.
14.1.3 Air-dry the control slides (see Section 13.3.3.12 for guidance).
14.2 Apply 50-µL of absolute methanol to each well containing the dried sample and allow to air-dry for 3 to 5 minutes.
14.3 Follow manufacturer's instructions in applying stain to slide.
14.4 Place the slides in a humid chamber in the dark and incubate at room temperature for approximately 30 minutes. The humid chamber consists of a tightly sealed plastic container containing damp paper towels on top of which the slides are placed.
14.5 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess detection reagent from below the well using a clean Pasteur pipette or absorb with paper towel or other absorbent material placed at edge of slide. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

14.6 Apply 50 µL of 4',6-diamidino-2-phenylindole (DAPI) staining solution (Section 7.7.2) to each well. Allow to stand at room temperature for a minimum of 1 minute. (The solution concentration may be increased up to 1 µg /mL if fading/diffusion of DAPI staining is encountered, but the staining solution must be tested first on expendable environmental samples to confirm that staining intensity is appropriate.)

14.7 Apply one drop of wash buffer (prepared according to the manufacturer's instructions [Section 7.6]) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess DAPI staining solution from below the well using a clean Pasteur pipette or absorb with paper towel or other absorbent material placed at edge of slide. Avoid disturbing the sample.

NOTE: If using the Merifluor stain (Section 7.6.1), do not allow slides to dry completely.

14.8 Add mounting medium (Section 7.8) to each well.
14.9 Apply a cover slip. Use a tissue to remove excess mounting fluid from the edges of the coverslip. Seal the edges of the coverslip onto the slide using clear nail polish.
14.10 Record the date and time that staining was completed on the bench sheet. If slides will not be read immediately, store in a humid chamber in the dark at 0 ^{o}C to 8 ^{o}C until ready for examination.

15.0 Examination
NOTE: Although immunofluorescence assay (FA) and 4',6-diamidino-2-phenylindole (DAPI) and differential interference contrast (DIC) microscopy examination and confirmation should be performed immediately after staining is complete, laboratories have up to 7 days from completion of sample staining to complete the examination and confirmation of samples. However, if fading/diffusion of FITC or DAPI staining is noticed, the laboratory must reduce this holding time. In addition the laboratory may adjust the concentration of the DAPI staining solution (Sections 7.7.2) so that fading/diffusion does not occur.

15.1 Scanning technique: Scan each well in a systematic fashion. An up-and-down or a side-to-side scanning pattern may be used (Figure 4).
15.2 Examination using immunofluorescence assay (FA), 4',6-diamidino-2-phenylindole (DAPI) staining characteristics, and differential interference contrast (DIC) microscopy. The minimum magnification requirements for each type of examination are noted below.
NOTE: All shape and measurements must be determined using 1000X magnification and reported to the nearest 0.5 µm.

Record examination results for Cryptosporidium oocysts on a Cryptosporidium report form; record examination results for Giardia cysts on a Giardia report form. All oocysts and cysts that meet the criteria specified in Sections 15.2.2 and 15.2.3, less atypical organisms specifically identified as non-target organisms by DIC or DAPI (e.g. possessing spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.), must be reported.
15.2.1 Positive and negative staining control.
15.2.1.1 Each analyst must characterize a minimum of three Cryptosporidium oocysts and three Giardia cysts on the positive staining control slide before examining field sample slides. This characterization must be performed by each analyst during each microscope examination session.

FITC examination must be conducted at a minimum of 200X total magnification, DAPI examination must be conducted at a minimum of 400X, and DIC examination must be conducted at a minimum of 1000X. Size, shape, and DIC and DAPI characteristics of the three Cryptosporidium oocysts and Giardia cysts must be recorded by the analyst on a microscope log. The analyst also must indicate on each sample report form whether the positive staining control was acceptable.
15.2.1.2 Examine the negative staining control to confirm that it does not contain any oocysts or cysts (Section 14.1). Indicate on each sample report form whether the negative staining control was acceptable.
15.2.1.3 If the positive staining control contains oocysts and cysts within the expected range and at the appropriate fluorescence for both FA and DAPI, and the negative staining control does not contain any oocysts or cysts (Section 14.1), proceed to Sections 15.2.2 and 15.2.3.

15.2.2 Sample examination—Cryptosporidium
15.2.2.1 FITC examination (the analyst must use a minimum of 200X total magnification). Use epifluorescence to scan the entire well for apple-green fluorescence of oocyst and cyst shapes. When brilliant apple-green fluorescing ovoid or spherical objects 4 to 6 µm in diameter are observed with brightly highlighted edges, increase magnification to 400X and switch the microscope to the UV filter block for DAPI (Section 15.2.2.2), then to DIC (Section 15.2.2.3).
15.2.2.2 DAPI examination (the analyst must use a minimum of 400X total magnification). Using the UV filter block for DAPI, the object will exhibit one of the following characteristics: (a) Light blue internal staining (no distinct nuclei) with a green rim (b) Intense blue internal staining (c) Up to four distinct, sky-blue nuclei Record oocysts in category (a) as DAPI negative; record oocysts in categories (b) and (c) as DAPI positive.
15.2.2.3 DIC examination (the analyst must use a minimum of 1000X total magnification). Using DIC, look for external or internal morphological characteristics atypical of Cryptosporidium oocysts (e.g., spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.) (adapted from Reference 20.6). If atypical structures are not observed, then categorize each apple-green fluorescing object as: (a) An empty Cryptosporidium oocyst (b) A Cryptosporidium oocyst with amorphous structure (c) A Cryptosporidium oocyst with internal structure (one to four sporozoites/oocyst)
Using 1000X total magnification, record the shape, measurements (to the nearest 0.5 µm), and number of sporozoites (if applicable) for each apple-green fluorescing object meeting the size and shape characteristics. Although not a defining characteristic, surface oocyst folds may be observed in some specimens.

NOTE: All measurements must be made at 1000X magnification.

15.2.3 Sample examination—Giardia
15.2.3.1 FITC examination (the analyst must use a minimum of 200X total magnification). When brilliant apple-green fluorescing round to oval objects (8 - 18 µm long by 5 - 15 µm wide) are observed, increase magnification to 400X and switch the microscope to the UV filter block for DAPI (Section 15.2.3.2) then to DIC (Section 15.2.3.3).
15.2.3.2 DAPI examination (the analyst must use a minimum of 400X total magnification). Using the UV filter block for DAPI, the object will exhibit one or more of the following characteristics: (a) Light blue internal staining (no distinct nuclei) and a green rim (b) Intense blue internal staining (c) Two to four sky-blue nuclei Record cysts in category (a) as DAPI negative; record cysts in categories (b) and (c) as DAPI positive.
15.2.3.3 DIC examination (the analyst must use a minimum of 1000X total magnification). Using DIC, look for external or internal morphological characteristics atypical of Giardia cysts (e.g., spikes, stalks, appendages, pores, one or two large nuclei filling the cell, red fluorescing chloroplasts, crystals, spores, etc.) (adapted from Reference 20.6). If atypical structures are not observed, then categorize each object meeting the criteria specified in Sections 15.2.3.1 - 15.2.3.3 as one of the following, based on DIC examination: (a) An empty Giardia cyst (b) A Giardia cyst with amorphous structure (c) A Giardia cyst with one type of internal structure (nuclei, median body, or axonemes), or (d) A Giardia cyst with more than one type of internal structure.
Using 1000X total magnification, record the shape, measurements (to the nearest 0.5 µm), and number of nuclei and presence of median body or axonemes (if applicable) for each apple-green fluorescing object meeting the size and shape characteristics.
NOTE: All measurements must be made at 1000X magnification.
15.2.4 Record the date and time that sample examination was completed on the report form.
15.2.5 Report Cryptosporidium and Giardia concentrations as oocysts/L and cysts/L.

16.0 Analysis of Complex Samples
16.1 Some samples may contain high levels (>1000/L) of oocysts and cysts and/or interfering organisms, substances, or materials. Some samples may clog the filter (Section 12.0); others will

not allow separation of the oocysts and cysts from the retentate or eluate; and others may contain materials that preclude or confuse microscopic examination.
16.2 If the sample holding time has not been exceeded and a full-volume sample cannot be filtered, dilute an aliquot of sample with reagent water and filter this smaller aliquot (Section 12.0). This dilution must be recorded and reported with the results.
16.3 If the holding times for the sample and for microscopic examination of the cleaned up retentate/eluate have been exceeded, the site should be re-sampled. If this is not possible, the results should be qualified accordingly.

17.0 Method Performance
17.1 Method acceptance criteria are shown in Tables 3 and 4 in Section 21.0. The initial and ongoing precision and recovery criteria are based on the results of spiked reagent water samples analyzed during the Information Collection Rule Supplemental Surveys (Reference 20.12). The matrix spike and matrix spike duplicate criteria are based on spiked source water data generated during the interlaboratory validation study of Method 1623 involving 11 laboratories and 11 raw surface water matrices across the U.S. (Reference 20.10).

NOTE: Some sample matrices may prevent the MS acceptance criteria in Tables 3 and 4 to be met. An assessment of the distribution of MS recoveries across 430 MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

18.0 Pollution Prevention
18.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.
18.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

19.0 Waste Management
19.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of these requirements can be found in the *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).
19.2 Samples, reference materials, and equipment known or suspected to have viable oocysts or cysts attached or contained must be sterilized prior to disposal.
19.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction,* both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

20.0 References

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20.11 "Dynabeads® GC-Combo," Dynal Microbiology R&D, P.O. Box 8146 Dep., 0212 Oslo, Norway (September 1998, Revision no. 01).
20.12 USEPA. Implementation and Results of the Information Collection Rule Supplemental Surveys. EPA-815-R-01-003. Office of Water, Office of Ground Water and Drinking Water, Standards and Risk Management Division, Washington, DC (2001).
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21.0 Tables and Figures
Table 1. Method Holding Times (See Section 8.2 for details)

Table 2. Tier 1 and Tier 2 Validation/Equivalency Demonstration Requirements

Test	Description	Tier 1 modification(1)	Tier 2 modification(2)
IPR (Section 9.4)	4 replicates of spiked reagent water	Required. Must be accompanied by a method blank.	Required per laboratory
Method blank (Section 9.6)	Unspiked reagent water	Required	Required per laboratory
MS (Section 9.5.1)	Spiked matrix water	Required on each water to which the modification will be applied and on every 20th sample of that water thereafter. Must be accompanied by an unspiked field sample collected at the same time as the MS sample	Not required
MS/MSD (Section 9.5)	2 replicates of spiked matrix water	Recommended, but not required. Must be accompanied by an unspiked field sample collected at the same time as the MS sample	Required per laboratory. Each laboratory must analyze a different water.

(1) If a modification will be used only in one laboratory, these tests must be performed and the results must meet all of the QC acceptance criteria in the method (these tests also are required the first time a laboratory uses the validated version of the method).
(2) If nationwide approval of a modification is sought for one type of water matrix (such as surface water), a minimum of 3 laboratories must perform the tests and the results from each lab individually must meet all QC acceptance criteria in the method. If more than 3 laboratories are used in a study, a minimum of 75% of the laboratories must meet all QC acceptance criteria.

NOTE: The initial precision and recovery and ongoing precision and recovery (OPR) acceptance criteria listed in Tables 3 and 4 are based on results from 293 Cryptosporidium OPR samples and 186 Giardia OPR samples analyzed by six laboratories during the Information Collection Rule Supplemental Surveys (Reference 20.12). The matrix spike acceptance criteria are based on data generated through interlaboratory validation of Method 1623 (Reference 20.10).

Table 3. Quality Control Acceptance Criteria for *Cryptosporidium*

Performance test	Section	Acceptance criteria
	9.4 9.4.2 9.4.2	
Initial precision and recovery Mean recovery (percent) Precision (as maximum relative standard deviation)		24 - 100 55
Ongoing precision and recovery (percent)	9.7	11 - 100
Matrix spike/matrix spike duplicate (for method modifications) Mean recovery1, 2(as percent) Precision (as maximum relative percent difference)	9.5 9.5.2 9.5.2	13 - 111 61

(1) The acceptance criteria for mean MS/MSD recovery serves as the acceptance criteria for MS recovery during routine use of the method (Section 9.5.1).
(2) Some sample matrices may prevent the acceptance criteria from being met. An assessment of the distribution of MS recoveries from multiple MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

Table 4.
Quality Control Acceptance Criteria for *Giardia*

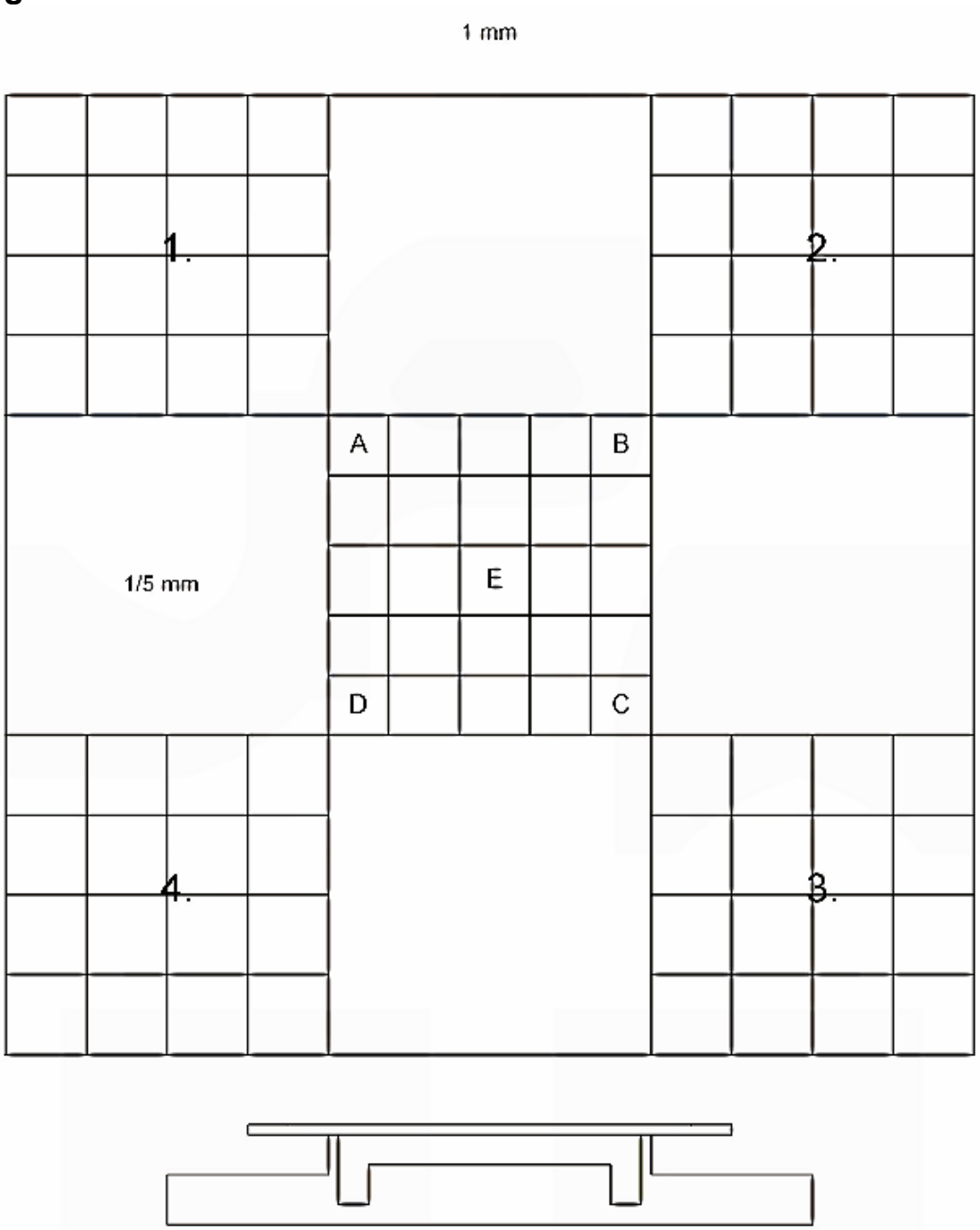
Quality Control Acceptance Criteria for *Giardia* Performance test	Section	Acceptance criteria
	9.4 9.4.2 9.4.2	
Initial precision and recovery Mean recovery (percent) Precision (as maximum relative standard deviation)		24 - 100 49
Ongoing precision and recovery (percent)	9.7	14 - 100
Matrix spike/matrix spike duplicate (for method modifications) Mean recovery* (as percent) Precision (as maximum relative percent difference)	9.5 9.5.2 9.5.2	15 - 118 30

(1) The acceptance criteria for mean MS/MSD recovery serves as the acceptance criteria for MS recovery during routine use of the method (Section 9.5.1).
(2) Some sample matrices may prevent the acceptance criteria from being met. An assessment of the distribution of MS recoveries across multiple MS samples from 87 sites during the ICR Supplemental Surveys is provided in Table 5.

Table 5. Distribution of Matrix Spike Recoveries from Multiple Samples Collected from 87 Source Waters During the ICR Supplemental Surveys (Adapted from Reference 20.13)

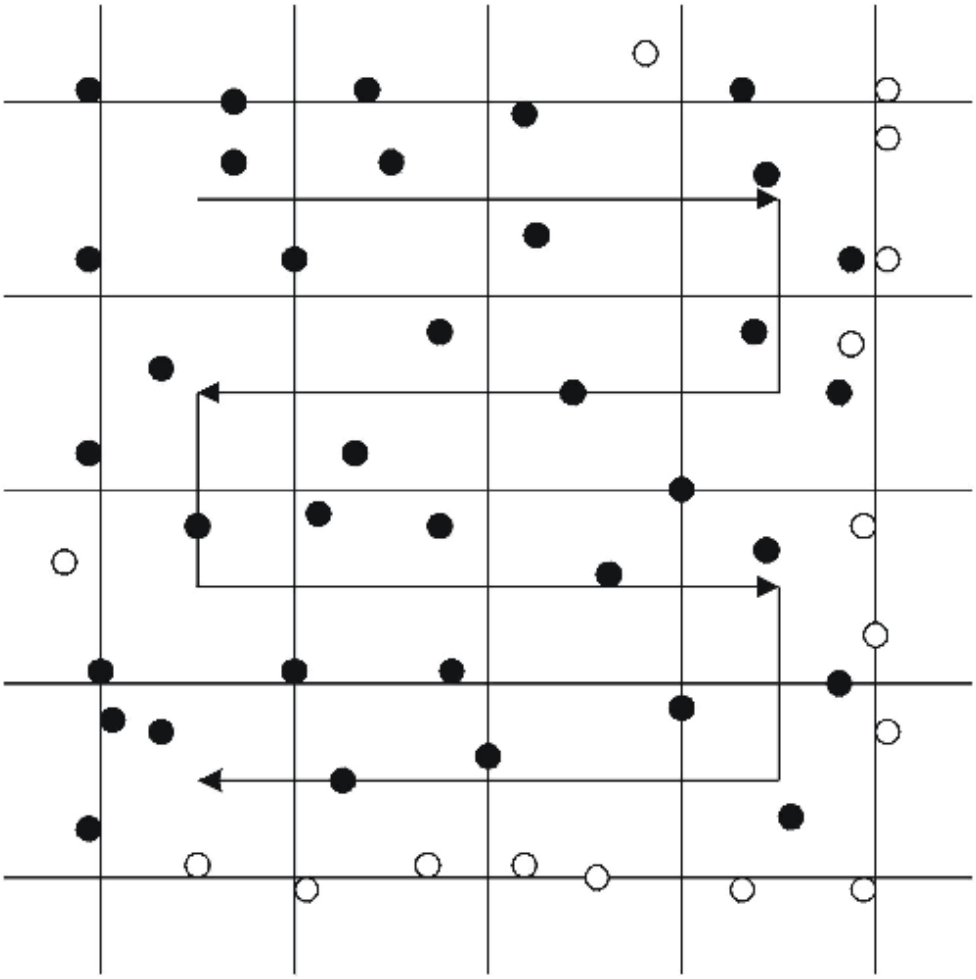
Source Waters During the ICR Supplemental Surveys (Adapted from Reference 20.13) MS Recovery Range	Percent of 430 *Cryptosporidium*MS Samples in Recovery Range	Percent of 270 *Giardia*MS Samples in Recovery Range
<10%	6.7%	5.2%
>10% - 20%	6.3%	4.8%
>20% - 30%	14.9%	7.0%
>30% - 40%	14.2%	8.5%
>40% - 50%	18.4%	17.4%
>50% - 60%	17.4%	16.3%
>60% - 70%	11.2%	16.7%
>70% - 80%	8.4%	14.1%
>80% - 90%	2.3%	6.3%
>90%	0.2%	3.7%

Figure 1.



Hemacytometer Platform Ruling. Squares 1, 2, 3, and 4 are used to count stock suspensions of *Cryptosporidium*oocysts and *Giardia* cysts (after Miale, 1967)

Figure 2.



Manner of Counting Oocysts and Cysts in 1 Square mm. Dark organisms are counted and light organisms are omitted (after Miale, 1967).

Figure 3. Laboratory Filtration System

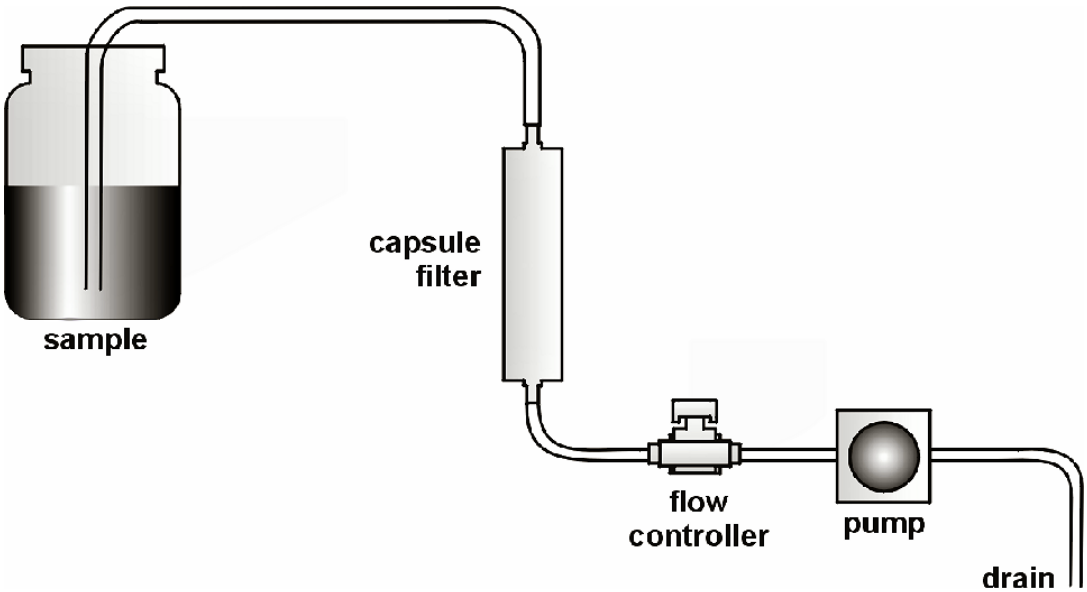
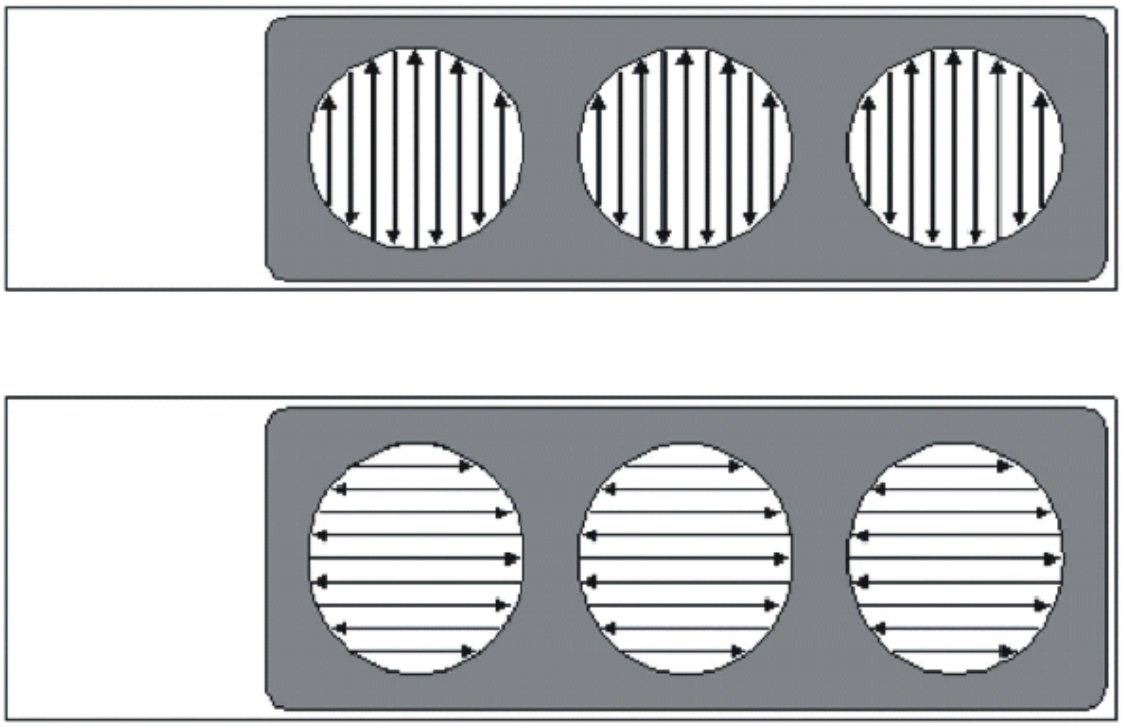


Figure 4. Methods for Scanning a Well Slide



Method 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)

1.0 Scope and Application

1.1 This test method describes a sensitive and differential membrane filter (MF) medium, using MI agar or MI broth, for the simultaneous detection and enumeration of both total coliforms (TC) and *Escherichia coli* (*E. coli*) in water samples in 24 hours or less on the basis of their specific enzyme activities. Two enzyme substrates, the fluorogen 4-Methylumbelliferyl- β -D-galactopyranoside (MUGal) and a chromogen Indoxyl-β-D-glucuronide (IBDG), are included in the medium to detect the enzymes β -galactosidase and β -glucuronidase, respectively, produced by TC and *E. coli*, respectively.

1.2 Total coliforms include species that may inhabit the intestines of warm-blooded animals or occur naturally in soil, vegetation, and water. They are usually found in fecally-polluted water and are often associated with disease outbreaks. Although they are not usually pathogenic themselves, their presence in drinking water indicates the possible presence of pathogens. *E. coli*, one species of the coliform group, is always found in feces and is, therefore, a more direct indicator of fecal contamination and the possible presence of enteric pathogens. In addition, some strains of *E. coli* are pathogenic (Reference 16.12).

1.3 This method, which has been validated for use with drinking water in single-lab and multi-lab studies (References 16.8 - 16.10), will be used primarily by certified drinking water laboratories for microbial analysis of potable water. Other uses include recreational, surface or marine water, bottled water, groundwater, well water, treatment plant effluents, water from drinking water distribution lines, drinking water source water, and possibly foods, pharmaceuticals, clinical specimens (human or veterinary), other environmental samples (*e.g.*, aerosols, soil, runoff, or sludge) and/or isolation and separation of transformants though the use of *E. coli lac Z* or *gus* A/*uid* reporter genes (Reference 16.11).

1.4 Since a wide range of sample volumes or dilutions can be analyzed by the MF technique, a wide range of *E. coli* and TC levels in water can be detected and enumerated.

2.0 Summary of Method

2.1 An appropriate volume of a water sample (100 mL for drinking water) is filtered through a 47-mm, 0.45-µm pore size cellulose ester membrane filter that retains the bacteria present in the sample. The filter is placed on a 5-mL plate of MI agar or on an absorbent pad saturated with 2-3 mL of MI broth, and the plate is incubated at 35°C for up to 24 hours. The bacterial colonies that grow on the plate are inspected for the presence of blue color from the breakdown of IBDG by the *E. coli* enzyme β -glucuronidase and fluorescence under long wave ultraviolet light (366 nm) from the breakdown of MUGal by the TC enzyme β -galactosidase (Reference 16.8).

3.0 Definitions

3.1 Total coliforms (TC) - In this method, TC are those bacteria that produce fluorescent colonies upon exposure to long wave ultraviolet light (366 nm) after primary culturing on MI agar or broth (See Figure 1.). The fluorescent colonies can be completely blue-white (TC other than *E. coli*) or blue-green (*E. coli*) in color or fluorescent halos may be observed around the edges of the blue-green E. coli colonies. In addition, non-fluorescent blue colonies, which rarely occur, are added to the total count because the fluorescence is masked by the blue color from the breakdown of IBDG (Reference 16.8).

3.2 Escherichia coli - In this method, the E. coli are those bacteria that produce blue colonies under ambient light after primary culturing on MI agar or broth (See Figures 1 and 2.). These colonies can be fluorescent or non-fluorescent under long wave ultraviolet light (366 nm) (Reference 16.8).

4.0 Interferences and Contamination

4.1 Water samples containing colloidal or suspended particulate material can clog the membrane filter, thereby preventing filtration, or cause spreading of bacterial colonies which could interfere

with identification of target colonies. However, the blue E. coli colonies can often be counted on plates with heavy particulates or high concentrations of total bacteria (See Figures 2 and 3.) (Reference 16.8).
4.2 The presence of some lateral diffusion of blue color away from the target E. coli colonies can affect enumeration and colony picking on plates with high concentrations of E. coli. This problem should not affect filters with low counts, such as those obtained with drinking water or properly diluted samples (Reference 16.8).
4.3 Tiny, flat or peaked pinpoint blue colonies (# 0.5-mm in diameter on filters containing # 200 colonies) may be due to species other than E. coli. These colonies occur occasionally in low numbers and should be excluded from the count of the E. coli colonies, which are usually much larger in size (1-3-mm in diameter). The small colonies have never been observed in the absence of typical E. coli, but, if such should occur, the sample should not be considered E. coli-positive unless at least one colony has been verified by another method [e.g., EC medium with 4-Methylumbelliferyl- β -D-glucuronide (MUG) or API 20E strips] (Reference 16.8).
4.4 Bright green, fluorescent, non-blue colonies, observed along with the typical blue/white or blue-green fluorescent TC colonies, may be species other than coliforms. These colonies, which generally occur in low numbers (# 5%) and can usually be distinguished from the TC, should be eliminated from the TC count. An increase in the number of bright green colonies may indicate an unusual sample population or a breakdown of the cefsulodin in the medium (Reference 16.8).

5.0 Safety
5.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and materials, and while operating sterilization equipment.
5.2 Mouth-pipetting is prohibited.
5.3 Avoid prolonged exposure to long wave or germicidal ultraviolet light.
5.4 Autoclave all contaminated plates and materials at the end of the analysis.

6.0 Equipment and Supplies
6.1 Incubator set at 35°C ± 0.5°C, with approximately 90% humidity if loose-lidded Petri dishes are used.
6.2 Stereoscopic microscope, with magnification of 10-15x, wide-field type.
6.3 A microscope lamp producing diffuse light from cool, white fluorescent lamps adjusted to give maximum color.
6.4 Hand tally.
6.5 Pipet container of stainless steel, aluminum, or Pyrex glass, for pipets.
6.6 Graduated cylinders (100-mL for drinking water), covered with aluminum foil or kraft paper and sterilized.
6.7 Membrane filtration units (filter base and funnel), glass, plastic or stainless steel. These are wrapped with aluminum foil or kraft paper and sterilized.
6.8 Germicidal ultraviolet (254 nm) light box for sanitizing the filter funnels is desirable, but optional.
6.9 Line vacuum, electric vacuum pump, or aspirator is used as a vacuum source. In an emergency, a hand pump or a syringe can be used. Such vacuum-producing devices should be equipped with a check valve to prevent the return flow of air.
6.10 Vacuum filter flask, usually 1 liter, with appropriate tubing. Filter manifolds to hold a number of filter bases are desirable, but optional.
6.11 Safety trap flask, placed between the filter flask and the vacuum source.
6.12 Forceps, straight (preferred) or curved, with smooth tips to permit easy handling of filters without damage.
6.13 Alcohol, 95% ethanol, in small wide-mouthed vials, for sterilizing forceps.
6.14 Bunsen or Fisher-type burner or electric incinerator unit.
6.15 Sterile T.D. (To Deliver) bacteriological or Mohr pipets, glass or plastic (1-mL and 10-mLvolumes).

6.16 Membrane Filters (MF), white, grid-marked, cellulose ester, 47-mm diameter, 0.45 µm ± 0.02-µm pore size, pretrial or sterilized for 10 minutes at 121°C (15-lb pressure).
6.17 Long wave ultraviolet lamp (366 nm), handheld 4-watt (preferred) or 6-watt, or microscope attachment.
6.18 Dilution water: Sterile phosphate-buffered dilution water, prepared in large volumes (e.g., 1 liter)for wetting membranes before addition of the sample and for rinsing the funnel after sample filtration or in 99-mL dilution blanks [Section 9050C in Standard Methods (Reference 16.2)].
6.19 Indelible ink marker for labeling plates.
6.20 Thermometer, checked against a National Institute of Science and Technology (NIST)-certified thermometer, or one traceable to an NIST thermometer.
6.21 Petri dishes, sterile, plastic, 9 x 50 mm, with tight-fitting lids, or 15 x 60 mm, glass or plastic, with loose-fitting lids; 15 x 100 mm dishes may also be used.
6.22 Bottles, milk dilution, borosilicate glass, screw-cap with neoprene liners, marked at 99 mL for 1:100 dilutions (if needed). Dilution bottles marked at 90 mL, or tubes marked at 9 mL may be used for 1:10 dilutions.
6.23 Flasks, borosilicate glass, screw-cap, 250- to 2000-mL volume, for agar preparation.
6.24 Waterbath maintained at 50°C for tempering agar.
6.25 Syringe filter, sterile, disposable, 25-mm diameter, 0.22-µm pore size, to filter cefsulodin for MI agar.
6.26 Syringe, sterile, plastic, disposable, 20-cc capacity. Autoclaved glass syringes are also acceptable.
6.27 Test tubes, sterile, screw-cap, 20 x 150-mm, borosilicate glass or plastic, with lids.
6.28 Sterilization filter units, presterile, disposable, 500- or 1000-mL capacity, 0.2-µm pore size, to filter stock buffer solutions.
6.29 Sterile 47-mm diameter absorbent pads (used with MI broth).
Note: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

7.0 Reagents and Standards
7.1 Purity of Reagents: Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (Reference 16.1). The agar used in preparation of culture media must be of microbiological grade.
7.2 Whenever possible, use commercial culture media as a means of quality control.
7.3 Purity of Water: Reagent-grade distilled water conforming to Specification D1193, Type II water or better, ASTM Annual Book of Standards (Reference 16.3).
7.4 Buffered Dilution Water (Reference 16.2)
7.4.1 Stock Phosphate Buffer Solution (Reference 16.2):
Potassium Dihydrogen Phosphate (KH_2PO_4) 34.0 g Reagent-Grade Distilled Water 500 mL
7.4.2 Preparation of Stock Buffer Solution: Adjust the pH of the solution to 7.2 with 1 N NaOH, and bring volume to 1000 mL with reagent-grade distilled water. Sterilize by filtration or autoclave for 15 minutes at 121°C (15-lb pressure).
7.4.3 $MgCl_2$ Solution (Reference 16.2): Dissolve 38 g anhydrous $MgCl_2$ (or 81.1 g $MgCl_2C6H_2O$) in one liter of reagent-grade distilled water. Sterilize by filtration or autoclave for 15 minutes at 121°C (15-lb pressure).
7.4.4 Storage of Stock Buffer and $MgCl_2$ Solutions: After sterilization of the stock solutions, store in the refrigerator until used. Handle aseptically. If evidence of mold or other contamination appears in either stock, the solution should be discarded, and a fresh solution should be prepared.
7.4.5 Working Solution (Final pH 7.0 ± 0.2): Add 1.25 mL phosphate buffer stock (Section 7.4.2) and 5 mL $MgCl_2$ stock (Section 7.4.3) for each liter of reagent-grade distilled water prepared. Mix well, and dispense in appropriate amounts for dilutions in screw-cap dilution bottles or culture tubes, and/or into larger containers for use as rinse water. Autoclave at 121°C (15-lb pressure) for

15 minutes. Longer sterilization times may be needed depending on the container and load size and the amount of time needed for the liquid to reach 121°C.

7.5 MI Agar (Reference 16.8)

7.5.1 Composition:

Proteose Peptone #3	5.0 g
Yeast Extract	3.0 g
β -D-Lactose	1.0 g
4-Methylumbelliferyl- β -D-Galactopyranoside (MUGal) (Final concentration 100µg/mL)	0.1 g
Indoxyl- β -D-Glucuronide (IBDG) (Final concentration 320 µg/mL)	0.32 g
NaCl	7.5 g
K_2HPO_4	3.3 g
KH_2PO_4	1.0 g
Sodium Lauryl Sulfate	0.2 g
Sodium Desoxycholate	0.1 g
Agar	15.0 g
Reagent-Grade Distilled Water	1000 mL

7.5.2 Cefsulodin Solution (1 mg / 1 mL): Add 0.02 g of cefsulodin to 20 mL reagent-grade distilled water, sterilize using a 0.22-µm syringe filter, and store in a sterile tube at 4°C until needed. Prepare fresh solution each time. Do not save the unused portion.

7.5.3 Preparation: Autoclave the medium for 15 minutes at 121°C (15-lb pressure), and add 5 mL of the freshly-prepared solution of Cefsulodin (5 µg/mL final concentration) per liter of tempered agar medium. Pipet the medium into 9 x 50-mm Petri dishes (5 mL/plate). Store plates at 4°C for up to 2 weeks. The final pH should be 6.95 ± 0.2.

7.6 MI Broth: The composition of MI broth is the same as MI agar, but without the agar. The final pH of MI broth should be 7.05 ± 0.2. The broth is prepared and sterilized by the same methods described for MI agar in Sections 7.5.1, 7.5.2, and 7.5.3, except that absorbent pads are placed in 9 x 50 mm Petri dishes and saturated with 2-3 mL of MI broth containing 5 :g/mL final concentration of Cefsulodin. Alternately, the broth can be filter-sterilized. Excess broth is poured off before using the plates. Plates should be stored in the refrigerator and discarded after 96 hours (Reference 16.15).

7.7 Tryptic Soy Agar/Trypticase Soy Agar (Difco 0369-17-6, BD 4311043, Oxoid CM 0129B, or equivalent) (TSA)

7.7.1 *Composition*:

Tryptone	15.0 g
Soytone	5.0 g
NaCl	5.0 g
Agar	15.0 g

7.7.2 *Preparation*: Add the dry ingredients listed above to 1000 mL of reagent-grade distilled water, and heat to boiling to dissolve the agar completely. Autoclave at 121°C (15-lb pressure) for 15 minutes. Dispense the agar into 9 x 50-mm Petri dishes (5 mL/plate). Incubate the plates for 24 - 48 hours at 35°C to check for contamination. Discard any plates with growth. If > 5% of the plates show contamination, discard all plates, and make new medium. Store at 4°C until needed. The final pH should be 7.3 ± 0.2.

8.0 Sample Collection, Preservation, and Storage

8.1 Water samples are collected in sterile polypropylene sample containers with leakproof lids.

8.2 Sampling procedures are described in detail in Sections 9060A and 9060B of the 18th edition of *Standard Methods for the Examination of Water and Wastewater* (Reference 16.2) or in the *USEPA Microbiology Methods Manual*, Section II, A (Reference 16.6). Residual chlorine in drinking water (or chlorinated effluent) samples should be neutralized with sodium thiosulfate (1 mL of a 10% solution per liter of water) at the time of collection. Adherence to sample preservation procedures and holding time limits are critical to the production of valid data. Samples not collected according to these rules should not be analyzed.

8.2.1 *Storage Temperature and Handling Conditions:* Ice or refrigerate water samples at a temperature of 1-4°C during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Take care that sample bottles are not totally immersed in water from melted ice during transit or storage.

8.2.2 *Holding Time Limitations:* Analyze samples as soon as possible after collection. Drinking water samples should be analyzed within 30 h of collection (Reference 16.13). Do not hold source water samples longer than 6 h between collection and initiation of analyses, and the analyses should be complete within 8 h of sample collection.

9.0 Calibration and Standardization

9.1 Check temperatures in incubators twice daily to ensure operation within stated limits (Reference 16.14).

9.2 Check thermometers at least annually against an NIST-certified thermometer or one traceable to NIST. Check mercury columns for breaks.

10.0 Quality Control (QC)

10.1 Pretest each batch of MI agar or broth for performance (*i.e.*, correct enzyme reactions) with known cultures (*E. coli*, TC, and a non-coliform).

10.2 Test new lots of membrane filters against an acceptable reference lot using the method of Brenner and Rankin (Reference 16.7).

10.3 Perform specific filtration control tests each time samples are analyzed, and record the results.

10.3.1 *Filter Control*: Place one or more membrane filters on TSA plates, and incubate the plates for 24 hours at 35°C. Absence of growth indicates sterility of the filter(s).

10.3.2 *Phosphate-Buffered Dilution Water Controls*: Filter a 50-mL volume of sterile dilution water before beginning the sample filtrations and a 50-mL volume of dilution water after completing the filtrations. Place the filters on TSA plates, and incubate the plates for 24 hours at 35°C. Absence of growth indicates sterility of the dilution water.

10.3.3 *Agar or Broth Controls*: Place one or more TSA plates and one or more MI agar plates or MI broth pad plates in the incubator for 24 hours at 35°C. Broth pad plates should be incubated *grid-side up*, not inverted like the agar plates. Absence of growth indicates sterility of the plates.

10.4 See recommendations on quality control for microbiological analyses in the "*Manual for the Certification of Laboratories Analyzing Drinking Water*: *Criteria and Procedures; Quality Assurance*" (Reference 16.15) and the *USEPA Microbiology Methods Manual*, part IV, C (Reference 16.6).

11.0 Procedure

11.1 Prepare MI agar or MI broth and TSA as described in Sections 7.5, 7.6, and 7.7. If plates are made ahead of time and stored in the refrigerator, remove them and allow them to warm to room temperature. The crystals that form on MI agar after refrigeration will disappear as the plates warm up (Reference 16.8).

11.2 Label the bottom of the MI agar or MI broth plates with the sample number/identification and the volume of sample to be analyzed. Label QC TSA plates and the MI agar or MI broth sterility control plate(s).

11.3 Using a flamed forceps, place a membrane filter, grid-side up, on the porous plate of the filter base. If you have difficulties in removing the separation papers from the filters due to static electricity, place a filter with the paper on top of the funnel base and turn on the vacuum. The separation paper will curl up, allowing easier removal.
11.4 Attach the funnel to the base of the filter unit, taking care not to damage or dislodge the filter. The membrane filter is now located between the funnel and the base.
11.5 Put approximately 30 mL of sterile dilution water in the bottom of the funnel.
11.6 Shake the sample container vigorously 25 times.
11.7 Measure an appropriate volume (100 mL for drinking water) or dilution of the sample with a sterile pipette or graduated cylinder, and pour it into the funnel. Turn on the vacuum, and leave it on while rinsing the funnel twice with about 30 mL sterile dilution water.
11.8 Remove the funnel from the base of the filter unit. A germicidal ultraviolet (254 nm) light box can be used to hold and sanitize the funnel between filtrations. At least 2 minutes of exposure time is required for funnel decontamination. Protect eyes from UV irradiation with glasses, goggles, or an enclosed UV chamber.
11.9 Holding the membrane filter at its edge with a flamed forceps, gently lift and place the filter grid-side up on the MI agar plate or MI broth pad plate. Slide the filter onto the agar or pad, using a rolling action to avoid trapping air bubbles between the membrane filter and the underlying agar or absorbent pad. Run the tip of the forceps around the outside edge of the filter to be sure the filter makes contact with the agar or pad. Reseat the membrane if non-wetted areas occur due to air bubbles.
11.10 Invert the agar Petri dish, and incubate the plate at 35°C for 24 hours. Pad plates used with MI broth should be incubated grid-side up at 35°C for 24 hours. If loose-lidded plates are used for MI agar or broth, the plates should be placed in a humid chamber.
11.11 Count all blue colonies on each MI plate under normal/ambient light, and record the results (See Figures 1 and 2.). This is the E. coli count. Positive results that occur in less than 24 hours are valid, but the results cannot be recorded as negative until the 24-hour incubation period is complete (Reference 16.14).
11.12 Expose each MI plate to long wave ultraviolet light (366 nm), and count all fluorescent colonies [blue/green fluorescent E. coli, blue/white fluorescent TC other than E. coli, and blue/green with fluorescent edges (also E. coli)] (See Figure 1.). Record the data.

11.13 Add any blue, non-fluorescent colonies (if any) found on the same plate to the TC count (Reference 16.8).

12.0 Data Analysis and Calculations
12.1 Use the following general rules to calculate the E. coli or TC per 100 mL of sample:

12.1.1 Select and count filters with # 200 total colonies per plate.

12.1.2 Select and count filter with # 100 target colonies (ideally, 20-80).

12.1.3 If the total number of colonies or TC on a filter are too-numerous-to-count or confluent, record the results as "TC^{+} (TNTC)" and count the number of E. coli. If both target organisms are $ 200, record the results as "TC^{+} EC^{+} (TNTC)".

12.1.4 Calculate the final values using the formula:

$$\text{E. coli/100 mL} = \frac{\text{Number of blue colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

$$\text{TC/100 mL} = \frac{\text{Number of fluorescent colonies + Number of blue, non-fluorescent colonies (if any)}}{\text{Volume of sample filtered (mL)}} \times 100$$

12.2 See the USEPA Microbiology Manual, Part II, Section C, 3.5, for general counting rules (Reference 16.6).
12.3 Report results as E. coli or TC per 100 mL of drinking water.

13.0 Method Performance

13.1 The detection limits of this method are one E. coli and/or one total coliform per sample volume or dilution tested (Reference 16.8).

13.2 The false-positive and false-negative rates for E. coli are both reported to be 4.3% (Reference 16.8).

13.3 The single lab recovery of E. coli is reported (Reference 16.8) to be 97.9% of the Heterotrophic Plate Count (pour plate) (Reference 16.2) and 115% of the R2A spread plate (Reference 16.2). For Klebsiella pneumoniae and Enterobacter aerogenes, two total coliforms, the recoveries are 87.5% and 85.7% of the HPC (Reference 16.8), respectively, and 89.3% and 85.8% of the R2A spread plate, respectively.

13.4 The specificities for *E. coli* and total coliforms are reported to be 95.7% and 93.1% (Reference 16.8), respectively.

13.5 The single lab coefficients of variation for *E. coli* and total coliforms are reported to be 25.1% and 17.6% (Reference 16.8), respectively, for a variety of water types.

13.6 In a collaborative study (References 16.4, 16.5, and 16.9), 19 laboratories concurrently analyzed six wastewater-spiked Cincinnati tap water samples, containing 3 different concentrations of *E. coli* (# 10, 11-30, and > 30 per 100 mL).

13.6.1 The single laboratory precision (coefficient of variation), a measure of the repeatability, ranged from 3.3% to 27.3% for *E. coli* and from 2.5% to 5.1% for TC for the six samples tested, while the overall precision (coefficient of variation), a measure of reproducibility, ranged from 8.6% to 40.5% and from 6.9% to 27.7%, respectively. These values are based on $\log_{10}$-transformed data (Reference 16.5).

13.6.2 Table 1 contains the statistical summary of the collaborative study (Reference 16.9) results.

14.0 Pollution Prevention

14.1 Pollution prevention is any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. It is the environmental management tool preferred over waste disposal or recycling. When feasible, laboratory staff should use a pollution prevention technique, such as preparation of the smallest practical volumes of reagents, standards, and media or downsizing of the test units in a method.

14.2 The laboratory staff should also review the procurement and use of equipment and supplies for other ways to reduce waste and prevent pollution. Recycling should be considered whenever practical.

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling releases from hoods and bench operations, complying with the letter and spirit of sewer discharge permits and regulations and by complying with solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. All infectious wastes should be autoclaved before disposal.

16.0 References

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16.4 American Society for Testing and Materials. 1994. Standard Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water, Designation D 2777-86, p. 31-44. In 1994 Annual Book of ASTM Standards, Section 11: Water and Environmental Technology, Volume 11.01. American Society for Testing and Materials, Philadelphia, PA.
16.5 Association of Official Analytical Chemists. 1989. Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis. Journal of the Association of Official Analytical Chemists 72 (4): 694-704.
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16.11 Buntel, C.J. 1995. E. coli $-Glucuronidase (GUS) as a Marker for Recombinant Vaccinia Viruses. BioTechniques 19 (3); 352-353.
16.12 Federal Register. 1985. National Primary Drinking Water Regulations; Synthetic Organic Chemicals, Inorganic Chemicals and Microorganisms; Proposed Rule. Federal Register 50: 46936-47022.
16.13 Federal Register. 1994. National Primary and Secondary Drinking Water Regulations: Analytical Methods for Regulated Drinking Water Contaminants; Final Rule. *Federal Register* 59: 62456-62471.
16.14 Federal Register. 1999. National Primary and Secondary Drinking Water Regulations: Analytical Methods for Chemical and Microbiological Contaminants and Revisions to Laboratory Certification Requirements; Final Rule. *Federal Register* 64: 67450-67467.
16.15 U.S. Environmental Protection Agency. 1992. Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures, Quality Assurance, Third Edition. EPA-814B-92-002, Office of Ground Water and Drinking Water, Technical Support Division, U.S. Environmental Protection Agency, Cincinnati, OH.

17.0 Tables and Figures

Table 1. Statistical Summary of the Collaborative Study Results[1]

Target Organism	Sample Number	*E. coli* Count Category (Range)[2]	Initial n[3]	Final n[4]	S_r[5]	RSD_r[6] (%)	$\bar{\chi}$[7]	S_R[8]	RSD_R[9] (%)	$\frac{RSD_R}{RSD_r}$ Ratio
Escherichia coli	1	Low (≤ 10)	63	63	0.17	27.3	0.64	0.26	40.5	1.49
	2		63	63	0.21	25.0	0.84	0.33	39.0	1.56
	3	Medium (11-30)	63	63	0.10	7.9	1.27	0.15	12.1	1.52
	4		63	60	0.07	5.6	1.32	0.12	9.2	1.65
	5	High (> 30)	63	60	0.06	3.3	1.87	0.16	8.6	2.62
	6		63	63	0.09	4.3	1.99	0.25	12.6	2.91
Total Coliforms	1	Low (≤ 10)	63	63	0.10	4.3	2.35	0.62	26.4	6.11
	2		63	63	0.09	3.8	2.31	0.64	27.7	7.25
	3	Medium (11-30)	63	63	0.11	5.1	2.17	0.47	21.8	4.28
	4		63	57	0.10	3.3	3.07	0.21	6.9	2.08
	5	High (> 30)	63	63	0.15	4.8	3.10	0.43	14.0	2.96
	6		63	63	0.08	2.5	3.14	0.46	14.7	5.97

[1] The values are based on $\log_{10}$ transformed data (Reference 16.5).

[2] The samples were grouped by their *E. coli* count on MI agar into the following categories:
Low (# 10 *E. coli* / 100 mL, samples 1 and 2),
Medium (11-30 *E. coli* / 100 mL, samples 3 and 4), and
High (> 30 *E. coli* / 100 mL, samples 5 and 6).

[3] These values are based on triplicate analyses by each laboratory. The reference laboratory analyzed three sets of samples: the initial and final samples prepared and a sample shipped along with the other 18 lab samples.

[4] These values were obtained after removing outliers by the AOAC procedure (Reference 16.5).

[5] S_r, Single Operator Standard Deviation, a measure of repeatability.

[6] RSD_r, Single Operator Relative Standard Deviation (Coefficient of Variance), a measure of repeatability.

[7] $\bar{\chi}$, The mean of the replicate analyses for all laboratories.

[8] S_R, Overall Standard Deviation, a measure of reproducibility.

[9] RSD_R, Overall Relative Standard Deviation (Coefficient of Variation), a measure of reproducibility.

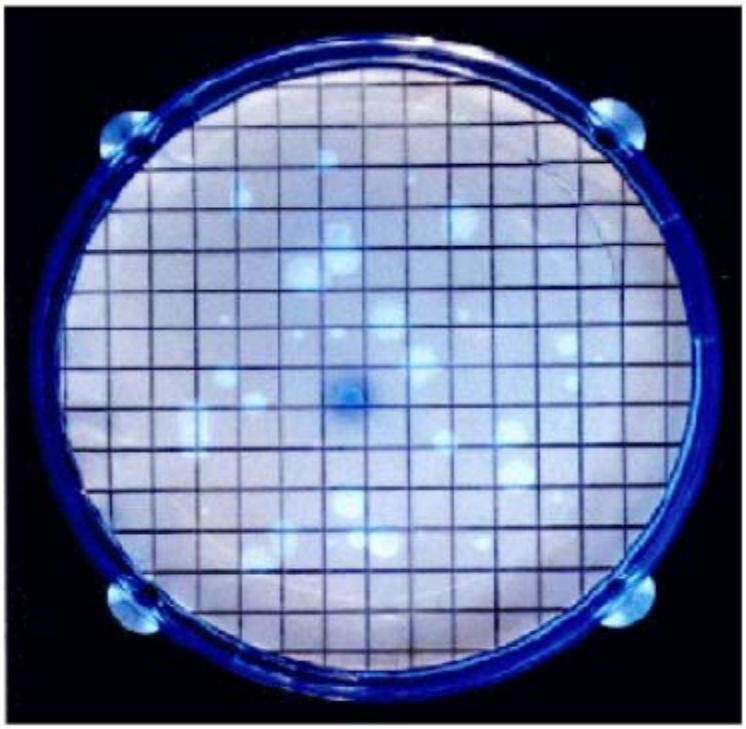

Figure 1. This photograph shows *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) on MI agar under long wave UV light (366 nm). The sample used was a wastewater-spiked Cincinnati, Ohio tap water.

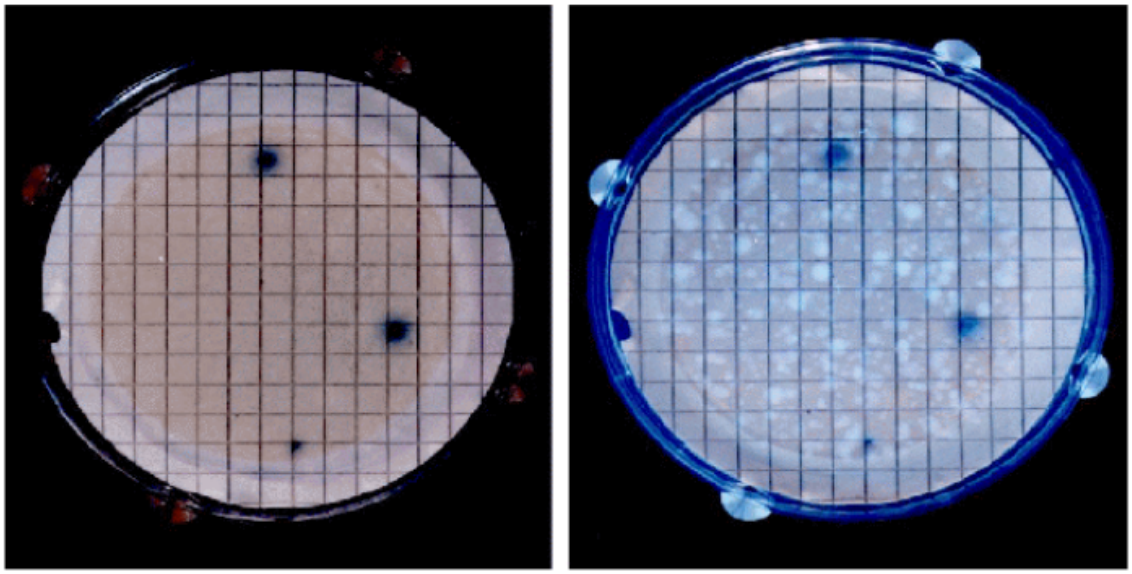

Figure 2. These photographs show *Escherichia coli* and total coliforms from cistern water on MI agar. The confluent plate was photographed under different lighting: ambient light on the left, and long wave UV light (366 nm) on the right. Under ambient light, *E. coli* are blue, and total coliforms other than *E. coli* and non-coliforms are their natural color. Under long wave UV light, all total coliforms, including *E. coli*, are fluorescent, and noncoliforms are non-fluorescent (*i.e.*, they are not visible).

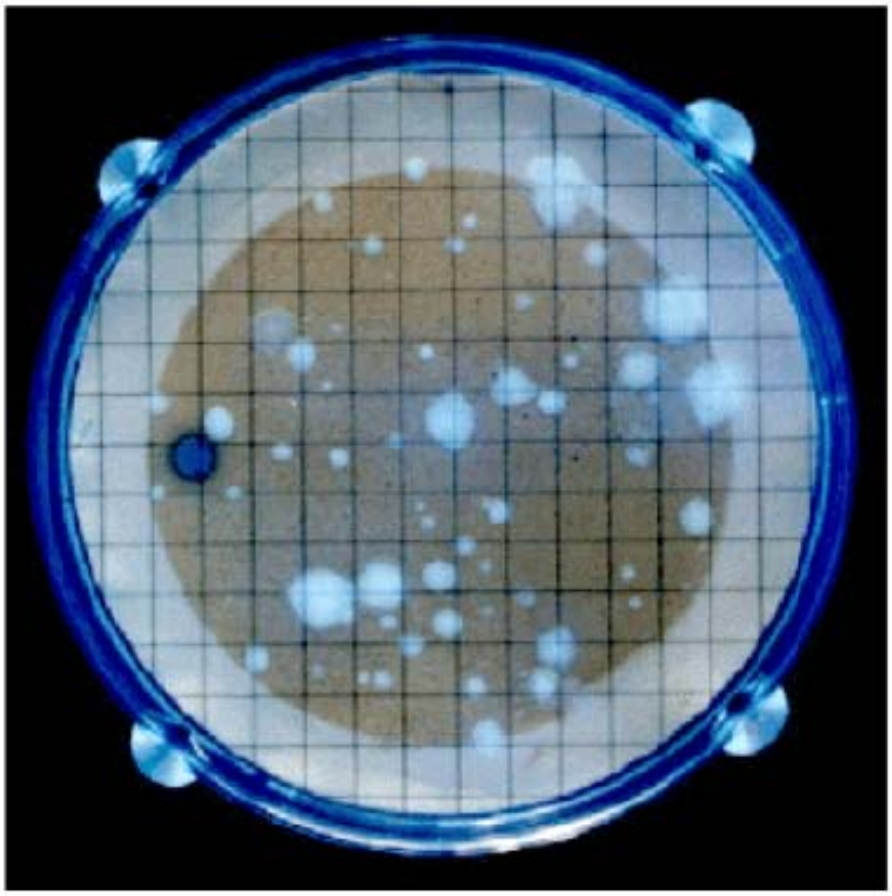

Figure 3. This photograph shows that *Escherichia coli* (blue/green fluorescence) and total coliforms other than *E. coli* (blue/white fluorescence) can easily be detected on MI agar plates from samples with high turbidity levels. The sample used was surface water-spiked Cincinnati, Ohio tap water.

Method 1605: *Aeromonas* in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V)

1.0 Scope and Application

1.1 This method describes a membrane filter (MF) procedure for the detection and enumeration of *Aeromonas* species in finished water samples. *Aeromonas* is a common genus of bacteria indigenous to surface waters. Its numbers are more likely to be greater during periods of warmer weather and when increased concentrations of organic nutrients are present. It is also more likely to be found in non-chlorinated water distribution systems or low-flow parts of chlorinated systems. Some *Aeromonas* species are opportunistic pathogens.

1.2 This method is adapted from Havelaar et al. (1987) for the enumeration of *Aeromonas* species in finished water by membrane filtration (Reference 15.1). It is a quantitative assay that uses a selective medium which partially inhibits the growth of non-target bacterial species while allowing *Aeromonas* to grow. *Aeromonas* is presumptively identified by the production of acid from dextrin fermentation producing yellow colonies. Presumptively positive colonies are counted and confirmed by testing for the presence of cytochrome *c* (oxidase test), and the ability to ferment trehalose, and produce indole.

1.3 This method is designed to meet the finished water monitoring requirements of the U.S. Environmental Protection Agency. *Aeromonas* was included on the Contaminant Candidate List (CCL) (Mar. 2, 1998, 63 *FR* 10274) and in the Revisions to the Unregulated Contaminant Monitoring Proposed Rule (UCMR) (September 17, 1999, 64 FR 50556). Contaminants listed in the UCMR are candidates for future regulation and may be included in a monitoring program for unregulated contaminants. Unregulated contaminant monitoring would be required for large systems and a representative sample of small and medium sized water distribution systems.

1.4 This method was subjected to an interlaboratory validation study involving 11 laboratories and 11 finished drinking water matrices. This method was not validated for other water types. Use of this method and appropriate validation for other water types is the responsibility of the user.

2.0 Summary of Method

2.1 The method provides a direct count of *Aeromonas* species in water based on the growth of yellow colonies on the surface of the membrane filter using a selective medium. A water sample is filtered through 0.45-Fm-pore-size membrane filter. The filter is placed on ampicillin-dextrin agar with vancomycin (ADA-V) and incubated at 35EC ± 0.5EC for 24 ± 2 hours. This medium uses ampicillin and vancomycin to inhibit non-*Aeromonas* species, while allowing most *Aeromonas* species to grow. The medium uses dextrin as a fermentable carbohydrate, and bromothymol blue as an indicator of acidity produced by the fermentation of dextrin. Presumptively identified yellow colonies are counted and confirmed by testing for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose and produce indole.

2.2 The membrane filtration procedure provides a direct count of culturable Aeromonas in water samples that is based on the growth of bacterial colonies on the surface of the membrane filter placed on a selective medium.

2.3 Aeromonas isolates may be archived for further analysis to determine species or hybridization group by inoculating a nutrient agar slant for short term use or shipping, or nutrient broth for freezing.

3.0 Definitions

3.1 Aeromonas are bacteria that are facultative anaerobes, Gram-negative, oxidase-positive, polarly flagellated, and rod shaped. They are classified as members of the family Aeromonadaceae. Demarta et al. (1999) reported 15 Aeromonas species based on 16S rDNA sequences though not all are officially recognized. Some species have been associated with human disease. In this method, Aeromonas are those bacteria that grow on ampicillin-dextrin agar with vancomycin (ADA-V), produce yellow colonies, are oxidase-positive, and have the ability to ferment trehalose and produce indole.

3.2 Definitions for other terms are provided in the glossary at the end of the method (Section 17.3).

4.0 Interferences and Contamination

4.1 Water samples containing colloidal or suspended particulate material may clog the membrane filter and prevent filtration or cause spreading of bacterial colonies which could interfere with identification of target colonies.

4.2 Other ampicillin/vancomycin resistant bacteria that are not aeromonads may be able to grow on this medium. Some of these bacteria may also produce yellow colonies if they are able to produce acid byproducts from the fermentation of dextrin or some other media component, or if they produce a yellow pigment. Enterococcus are reported to produce pinpoint-size yellow colonies on ADA. Confirmation of presumptive Aeromonas colonies is necessary to mitigate false positives.

5.0 Safety

5.1 Some strains of Aeromonas are opportunistic pathogens. Sample containers and waste materials should be autoclaved prior to cleaning or disposal.

5.2 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents, and other materials.

5.3 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies

Note: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Equipment for collection and transport of samples to laboratory

- **6.1.1** Autoclavable sample container—Use sterile, non-toxic, glass or plastic containers with a leak-proof lid. Ensure that the sample container is capable of holding a 1-L sample with ample headspace to facilitate mixing of sample by shaking prior to analysis.
- **6.1.2** Ice chest
- **6.1.3** Ice packs

6.2 Autoclavable dilution bottles—125-mL marked at 99 mL or 90 mL; commercially produced dilution bottles may be used.

6.3 Rinse water bottles

6.4 Sterile plastic or autoclavable glass pipettes with a 2.5% tolerance—to deliver (TD), 1- and 10-mL

6.5 Pipette bulbs or automatic pipetter.

6.6 Autoclavable pipette container (if using glass pipettes).

6.7 Thermometer—with 0.5EC gradations checked against a National Institute of Standards and Technology (NIST) certified thermometer, or one that meets the requirements of NIST Monograph SP 250-23.

6.8 Inoculating loop—Sterile metal, plastic, or wooden applicator sticks.

6.9 Burner—Flame or electric incinerator for sterilizing metal inoculating loops and forceps.

6.10 Colony counting device—Mechanical, electric or hand tally.

6.11 Hotplate stirrer

6.12 Magnetic stir bar

6.13 Graduated cylinders—100 mL, 500 mL and 1 L, sterile, polypropylene or glass

6.14 Balance—Capable of weighing samples up to 200 g, with a readability of 0.1 g

6.15 Weigh boats

6.16 pH meter

6.17 Turbidimeter (optional)

6.18 Equipment for membrane filter procedure

6.18.1 Incubator—Hot air or water-jacketed microbiological type to maintain a temperature of 35EC ± 0.5EC
6.18.2 Petri dishes—sterile, 50 × 9 mm or other appropriate size
6.18.3 Membrane filtration units (filter base and funnel made of glass, plastic, or stainless steel), wrapped with aluminum foil or Kraft paper, and sterilized by autoclaving.
6.18.4 Vacuum source
6.18.5 Flasks—1-L vacuum filter with appropriate tubing; a filter manifold to hold a number of units is optional
6.18.6 Side-arm flask to place between vacuum source and filtration devices or filter manifold
6.18.7 Membrane filters—Sterile, cellulose ester, white, gridded, 47-mm-diameter with 0.45-Fm pore size (Gelman E04WG04700 or equivalent)
6.18.8 Forceps—Sterile, straight or curved, with smooth tips to handle filters without causing damage
6.18.9 Ethanol or other alcohol in a container to sterilize forceps
6.18.10 Test tubes—125 × 16 mm sterile, screw-cap tube
6.19 Dissecting microscope—Low power (10X to 15X), binocular, illuminated
6.20 Autoclave—Capable of 121EC at 15 psi. Must meet requirements set forth in the Manual for the Certification of Laboratories Analyzing Drinking Water, 4th Edition. (Reference 15.5)
6.21 Membrane filters (for sterilization purposes)—Sterile with 0.22-Fm pore size (Gelman Acrodisc No. 4192 or equivalent)

7.0 Reagents and Standards
7.1 Purity of reagents and culture media—Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, reagents and culture media shall conform to the specifications in Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141), Section 9050 (Reference 15.2). The agar used in preparation of culture media must be of microbiological grade.
7.2 Purity of water—Reagent-grade water conforming to specifications in Manual for the Certification of Laboratories Analyzing Drinking Water, 4th Edition (Reference 15.5) or Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141), Section 9020 (Reference 15.2).

7.3 Phosphate buffered dilution water
7.3.1 Concentrated stock phosphate buffer solution—Dissolve 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 mL reagent-grade water. Adjust the pH to 7.2 ± 0.5 with 1N sodium hydroxide (NaOH) and dilute to 1 L with reagent-grade water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.
7.3.2 Magnesium chloride solution—Dissolve 81.1 g magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_20$) in reagent-grade water and dilute to 1 L. Autoclave or filter sterilize through a 0.22-Fm-pore-size filter.
7.3.3 Prepare phosphate buffered dilution water by adding 1.25 mL of concentrated stock phosphate buffer solution (Section 7.3.1) and 5.0 mL of magnesium chloride solution (Section 7.3.2) to a 1-L graduated cylinder and adjust final volume to 1 L with reagent-grade water. Prepare a portion of buffered dilution water in 1-L bottles for rinse water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.
7.3.4 Stored phosphate buffered dilution water should be free from turbidity.
7.4 Ampicillin-dextrin agar with vancomycin (ADA-V)
7.4.1 Preparation of dextrin agar—EPA highly recommends the use of commercial ADA (m-Aeromonas Selective Agar Base [Havelaar]), Section **7.4.1.1**. However, ADA may be prepared by the laboratory (Section 7.4.1.2)
7.4.1.1 Commercial dextrin agar—Tech Pac (distributor, tech@fuse.net), Cincinnati, Ohio; Biolife (www.biolifeit.com) Italiana Srl, 272 Viale Monza, Milan, Italy, Cat. No. 401019 or equivalent. Prepare 1-L of media, according to manufacturer's instructions. Cool to room temperature, and adjust

pH to 8.0 using 1N NaOH or 1N HCl. Autoclave for 15 min, cool to 50EC.

7.4.1.2 Laboratory-prepared dextrin agar.

7.4.1.2.1 5.0 g tryptose—Difco cat. no. 0124-17, or equivalent

7.4.1.2.2 11.4 g dextrin—Difco cat. no. 0161-17, or equivalent

7.4.1.2.3 2.0 g yeast extract—Difco cat. no. 0127-17, or equivalent

7.4.1.2.4 3.0 g sodium chloride (NaCl)—Baker cat. no. 3624, or equivalent

7.4.1.2.5 2.0 g potassium chloride (KCl)—Fisher cat. no. P217-500, or equivalent

7.4.1.2.6 0.1 g magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)—Fishercat. no. M63-500, or equivalent

7.4.1.2.7 0.06 g ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$)—Sigma cat. no. F-2877, or equivalent

7.4.1.2.8 0.08 g bromothymol blue—Baker cat. no. D470, or equivalent

7.4.1.2.9 Sodium deoxycholate—Sigma cat. no. D-6750, or equivalent. Add 100 mg of sodium deoxycholate to 10 mL of reagent water.

7.4.1.2.10 13.0 g agar, bacteriological grade—Fisher cat. no. BP1423-500, or equivalent.

7.4.1.2.11 Add reagents in Sections 7.4.1.2.1 through 7.4.1.2.8 to 1-L of reagent-grade water, stir to dissolve and adjust pH to 8.0 using1N NaOH or 1N HCl. After the pH has been adjusted, add sodium deoxycholate (Section 7.4.1.2.9) and agar (Section7.4.1.2.10) and heat to dissolve. Autoclave for 15 min, cool to 50°C.

7.4.2 Ampicillin, sodium salt—Sigma cat. no. A0166, or equivalent. Add 10 mg of ampicillin, sodium salt to 10 mL reagent water. Prepare on the same day that medium is prepared and filter sterilize through a 0.22-Fm-pore-size filter. Alternatively, use Biolife cat. no. 4240012 prepared according to manufacturer's instructions, taking care to use an appropriate amount of ampicillin for the volume of media being prepared (for example, use two vials for a 1-L batch of ADA-V). Follow manufacturer's instructions
for appropriate storage temperature and shelf-life. Wear suitable protective clothing, gloves, and eye/face protection and prepare stock solutions in a chemical fume hood.

7.4.3 Vancomycin hydrochloride—Sigma cat. no. V2002, or equivalent. Add 2 mg of vancomycin hydrochloride to 10 mL of reagent water. Filter sterilize through a 0.22-Fm-pore-size filter. Follow manufacturer's instructions for appropriate storage temperature and time. Wear suitable protective clothing, gloves, and eye/face protection and prepare stock solutions in a chemical fume hood.

7.4.4 After dextrin agar (Section 7.4.1) has been autoclaved and cooled to 50EC, add the sterile ampicillin (Section 7.4.2) and sterile vancomycin hydrochloride solutions (Section 7.4.3).

7.4.5 Add approximately 5 mL of ADA-V per 50 × 9 mm Petri dish and allow to solidify. For larger plates, adjust volume appropriately. ADA-V plates should be stored in a tight fitting container (i.e. sealed plastic bag) at a temperature of 1EC to 5EC for no longer than 14 days.

7.5 Pentahydrate ACS Reagent grade sodium thiosulfate—Fisher cat. no. S445-500, or equivalent. Prepare a 3% stock solution by adding 3 g sodium thiosulfate to 100 mL reagent-grade water.

7.6 Disodium salt of ethylenediaminetetraacetic acid (EDTA)—Sigma cat. no. E 4884, or equivalent. EDTA should only be added to samples if metals in water samples exceed 1.0 mg/L. To prepare stock solution, add 12.4 g EDTA to 80 mL of reagent-grade water. Adjust pH to 8.0 using 10N NaOH. After the pH has been adjusted, bring the volume up to 100 mL with reagent-grade water.

7.7 Positive control culture—Aeromonas hydrophila ATCC #7966; obtained from the American Type Culture Collection (ATCC, 10801 University Blvd, Manassas, VA, 20110-2209; http://www.atcc.org).

7.8 Negative culture control—Negative culture controls serve two purposes: to ensure the laboratories are familiar with the color and morphology of non-Aeromonas bacteria that may grow on ADA-V and to ensure that confirmation test results are appropriate. E. coli (ATCC #25922) is the negative culture control for oxidase, Pseudomonas aeruginosa (ATCC #27853) is the

negative culture control for trehalose fermentation, and Bacillus cereus (ATCC #11778) is the negative culture control for indole.

7.9 Nutrient agar—Difco cat. no. 0001-17 or equivalent. Prepare according to manufacturer's instructions.

7.10 Oxidase reagents—Dry Slide BBL cat. no. 231746 or equivalent.

7.11 0.5% Trehalose confirmation reagent

7.11.1 Add 5 g trehalose (Sigma cat. no. T0167, or equivalent) to 100 mL water and filter sterilize solution through a filter with 0.22-Fm-pore-size.

7.11.2 Prepare 900 mL purple broth base (Difco cat. no. 0222-17, or equivalent) according to manufacturer's instructions and autoclave.

7.11.3 Aseptically add 100 mL trehalose solution to the cooled 900 mL of purple broth base.

7.11.4 Dispense into 6 mL or larger size tubes and fill approximately half full. Store in refrigerator.

Note: *Alternatively, prepare purple broth base according to manufacturer's instructions, add 5 g trehalose per liter, and filter sterilize through a filter with 0.22-Fm-pore-size.*

7.12 Tryptone broth—Oxoid cat. no. CM0087B, or equivalent. Alternatively, the laboratory may prepare tryptone broth by adding 10 g of tryptone (Difco cat. no. 0123-17 or equivalent) and 5 g of NaCl to 1 L of reagent water. Autoclave or filter sterilize through a filter with 0.22-Fm-pore-size.

7.13 Kovac's reagent—Biomeriuex cat. no. V7050, or equivalent

8.0 Sample Collection, Preservation, and Storage

8.1 Adherence to sample preservation procedures and holding time limits specified in *Standard Methods for the examination of Water and Wastewater* (Reference 15.2) is critical to the production of valid data. Sample results will be considered invalid if those conditions are not met.

8.2 Preparation of sample bottles and sample collection—Samples must be representative of the drinking water distribution system. Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. Cold water taps should be used. The service line should be cleared before sampling by maintaining a steady water flow for at least two minutes (until the water changes temperature).

8.2.1 Use sterile, non-toxic, glass or plastic container (Section 6.1.1) with a leak-proof lid. Ensure that the sample container is capable of holding a 1-L sample with ample headspace to facilitate mixing of sample by shaking prior to analysis. Sampling procedures are described in detail in *Standard Methods for the Examination of Water and Wastewater*, Section 9060 (Reference 15.2).

8.2.2 Add 1 mL of 3% sodium thiosulfate stock (Section 7.5) per L of sample to sample bottles prior to autoclave sterilization. Alternatively, if using presterilized sample bottles, sodium thiosulfate should be autoclaved for 15 minutes or filter sterilized through a filter with 0.22-Fm-pore-size before adding to the sample bottles.

8.2.3 If metals in the sample exceed 1.0 mg/L, add 3 mL of EDTA stock solution (Section 7.6) per L of sample to sample bottles prior to autoclave sterilization. If using presterilized sample bottles, EDTA should be autoclaved for 15 minutes or filter sterilized through a filter with 0.22-Fm-pore-size.

8.2.4 Collect a minimum of 1-L of sample.

8.3 Sample preservation and handling

8.3.1 Immediately following sample collection, tighten the sample container lid(s) and place the sample container(s) upright in an insulated, plastic-lined storage cooler with ice packs or in a refrigerator to chill prior to packing the cooler for shipment. Do not freeze the sample.

8.3.2 Use enough solidly frozen ice packs to ensure that the samples will arrive at a temperature of 1°C to 10°C. Use a minimum of two ice packs per shipment and add extra ice packs for multiple samples. Place one or more ice packs on each side of the container to stabilize samples.

8.3.3 Samples must be maintained at a temperature of 1°C to 10°C during shipment. Samples must not be frozen.

Note: Sample temperature during shipment is critical. Ice packs must be frozen solid immediately prior to shipment.

8.4 Verify and record sample arrival temperature when received in the laboratory. Refrigerate samples at 1°C to 5°C upon receipt at the laboratory and analyze as soon as possible after collection. Samples must be analyzed within 30 hours of sample collection.

9.0 Quality Control

9.1 Each laboratory that uses Method 1605 is required to operate a formal quality assurance (QA) program. The minimum QA requirements consist of the initial demonstration of capability (IDC) test (Section 9.4), ongoing analysis of spiked reagent water (ODC test, Section 9.8) and spiked finished drinking water samples (MS/MSD, Section 9.7), and analysis of negative culture controls (Section 9.6), dilution/rinse water blanks (Section 9.5), and media sterility checks (Section 9.2.6) as tests of continued acceptable performance. Spiked sample results are compared to acceptance criteria for precision, which are based on data generated during the interlaboratory validation of Method 1605 involving 11 laboratories and 11 finished water matrices. The more stringent QA requirements in this method, relative to other, currently used methods for bacterial determination, are an effort to improve overall microbiological QA. The specifications contained in this method can be met if the analytical system is maintained under control. Laboratories are not permitted to modify ADA-V media or procedures associated with filtration (Sections 10.1 through 10.10). However, the laboratory is permitted to modify method procedures related to the confirmation of colonies (Section 10.11) to improve performance or lower the costs of measurements provided that 1) presumptively identified yellow colonies submitted to confirmation are tested for the presence of cytochrome c (oxidase test), and the ability to ferment trehalose, and the ability produce indole, and 2) all quality control (QC) tests cited in Section 9.2.12 are performed acceptably and QC acceptance criteria are met. For example, laboratories may prefer to streak colonies that are submitted to confirmation on tryptic soy agar (TSA), instead of nutrient agar. The laboratory may not omit any quality control analyses.

9.2 General QC requirements—Specific quality control (QC) requirements for Method 1605 are provided below. QA and QC criteria for facilities, personnel, and laboratory equipment, instrumentation, and supplies used in microbiological analyses must be followed according to Standard Methods for the Examination of Water and Wastewater (latest edition approved by EPA in 40 CFR Part 141, Reference 15.2) and the U.S. EPA Manual for the Certification of Laboratories Analyzing Drinking Water, Fourth Edition (March 1997) (Reference 15.5).

9.2.1 Initial demonstration of capability (IDC). The laboratory shall demonstrate the ability to generate acceptable performance with this method by performing an IDC test before analyzing any field samples. The procedure for performing the IDC is described in Section 9.4. IDC tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2), negative culture controls (Section 9.2.3), and media sterility checks (Section 9.2.6).

9.2.2 Dilution/rinse water blanks. The laboratory shall analyze dilution/rinse water blanks to demonstrate freedom from contamination. The procedures for analysis of dilution/rinse water blanks are described in Section 9.5. At a minimum, dilution/rinse water blanks must be processed at the beginning and end of each filtration series to check for possible cross-contamination. A filtration series ends when 30 minutes or more elapse between sample filtrations. An additional dilution/rinse water blank is also required for every 20 samples, if more than 20 samples are processed during a filtration series.

9.2.3 Negative culture controls. The laboratory shall analyze negative culture controls (Section 9.6) to ensure that ADA-V and the confirmation procedures are performing properly. Negative culture controls should be run whenever a new batch of media or reagents is used. On an ongoing basis, the laboratory must perform, at a minimum, one negative culture control per week during weeks the laboratory analyzes field samples.

9.2.4 Matrix spike/matix spike duplicate (MS/MSD). The laboratory shall analyze one set of MS/MSD samples when samples are first received from a finished drinking water source for which the laboratory has never before analyzed samples (Section 9.7). Subsequently, 5% of field samples from a given source must include an MS/MSD test. Additional MS/MSD tests are also

recommended when drinking water treatment is adjusted or when other events take place, for example, when scrubbing or replacing lines. When possible, MS/MSD analyses should be conducted on the same day as ODC samples, using the same spiking procedure and volume.
9.2.4.1 Precision. MS/MSD sample results should meet the precision criteria set forth in Section 12, Table 1.
9.2.4.2 Recovery. QC acceptance criteria for Aeromonas recovery are not included in this method because the number of Aeromonas in the spike is unknown. However, each laboratory should control chart the mean number of Aeromonas per MS/MSD set (adjusted for background) and maintain a record of spike preparation procedures and spike volume. The laboratory should compare number of Aeromonas in MS/MSD samples to results of ODC samples (Section 9.2.5 and 9.8) spiked on the same day. This comparison should help the laboratory recognize when a matrix is interfering with method recovery. If the laboratory observes consistent ODC results from week to week, control charting the MS/MSD results by source may also help to recognize fluctuations in recovery from a particular source.

9.2.5 Ongoing demonstration of capability (ODC). The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate, Section 9.8).
9.2.5.1 Frequency. The laboratory shall analyze one set of ODC samples after every 20 field and MS samples or one set per week that samples are analyzed, whichever occurs more frequently. No more than one set of ODC samples is required per day, provided that the same equipment (i.e., incubators) are being used for all the samples.
9.2.5.2 Precision. ODC sample results must meet the precision criteria set forth in Section 12, Table 1.
9.2.5.3 Recovery. QC acceptance criteria for Aeromonas recovery are not included in this method because the initial spike dose for ODC samples is unknown.
As a result, each laboratory should control chart the mean number of Aeromonas per ODC sample set and maintain a record of spike preparation procedures and ODC spike volume. Maintaining this information will enable the laboratory to recognize when problems arise. Example: A laboratory that prepares spiking suspensions according to Section 9.3, spikes QC samples with 5 mL of dilution D2, and typically recovers approximately 50 Aeromonas per sample, and maintains a control chart of these counts. If the laboratory continues to prepare spiking suspensions the same way, but the number of Aeromonas counted declines noticeably (e.g. 20 Aeromonas per sample), then there may be a problem with the media, reagents, or the spiking suspension.
9.2.6 Media sterility checks. The laboratory shall test media sterility by incubating one unit (tube or plate) from each batch of medium (ADA-V, nutrient agar slant, nutrient agar, streak plate, trehalose, and tryptone) at 35°C ± 0.5°C for 24 ± 2 hours and observing for growth.
9.2.7 Analyst colony counting variability. If the laboratory has two or more analysts, each are required to count target colonies on the same membrane from one ODC sample per month (Section 9.9), at a minimum.
9.2.8 Record maintenance. The laboratory shall maintain records to define the quality of data that are generated. The laboratory shall maintain a record of the date and results of all QC sample analyses described in Section 9.2. A record of media sterility check, dilution/rinse water blank, analyst counting variability, IDC, ODC, and MS/MSD sample results must be maintained. Laboratories shall maintain reagent and material lot numbers along with samples analyzed using each of the lots. Laboratories shall also maintain media preparation records.
9.2.9 Performance studies. The laboratory should periodically analyze external QC samples, such as performance evaluation (PE) samples, when available. The laboratory should also participate in available interlaboratory performance studies conducted by local, state, and federal agencies or commercial organizations. The laboratory should review results, correct unsatisfactory performance, and record corrective actions.
9.2.10 Autoclave sterilization verification. At a minimum, the laboratory shall verify autoclave sterilization according to the procedure in Section 9.10 on a monthly basis.
9.2.11 Culture maintenance. The laboratory should use 24 ± 2 hour-old nutrient agar slant cultures for preparation of IDC, ODC, and MS/MSD spiking suspension dilutions. The laboratory

should use 22 to 72 hour-old nutrient agar slant cultures to inoculate ADA-V streak plates for analysis of negative culture controls. With regard to the preparation of subcultures, it is recommended that a maximum of three passages be prepared to help avoid contamination. After three passages, start a new subculture from the frozen stock.

9.2.12 Method modification.

9.2.12.1 **Membrane filtration**. Because recovery criteria are not available for this method, laboratories are not permitted to modify the membrane filtration procedures (Section 10.1 through Section 10.10.) or ADA-V media.

9.2.12.2 **Confirmation procedures**. The confirmation procedures in Section 10.11 may be modified, provided that the laboratory demonstrates the ability to generate acceptable performance by performing an IDC test (Section 9.2.1) and the appropriate negative culture control test(s) (Section 9.2.3) before analyzing any field samples using the modified confirmation. 100% of the colonies submitted to confirmation from IDC and negative culture control samples must give the appropriate confirmation response. These tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2) and media sterility checks (Section 9.2.6).

9.3 Preparation of Aeromonas spiking suspension for use in spiking IDC, ODC, and MS/MSD samples—This dilution scheme is adapted from Standard Methods for the Examination of Water and Wastewater, 19th Edition, Section 9020 B (Reference 15.9). This entire process should be performed quickly to avoid loss of viable organisms. See Section 16, Flowchart 1, for an example of this dilution scheme. Please note: Provided that all QC acceptance criteria are met and the recommended target range of 20 - 60 CFU per plate are typically observed, laboratories may prepare QC spiking suspensions using commercial products or other procedures such as growing bacteria in a broth, measuring optical density, and spiking each test sample with an equivalent volume.

9.3.1 Inoculate Aeromonas hydrophila (ATCC #7966) onto the entire surface of several nutrient agar slants with a slope approximately 6.3 cm long in a 125 × 16 mm screw-cap tube. Incubate for 24 ± 2 hours at 35°C ± 0.5°C.

9.3.2 From the slant that has the best growth, prepare serial dilutions using four dilution bottles with 99 mL of sterile buffered dilution water (A, B, C and D below in Sections 9.3.3 and 9.3.4) and one dilution bottle containing 90-mL of sterile buffered dilution water (D2 below in Section 9.3.5).

9.3.3 Pipette 1 mL of buffered dilution water from bottle “A” to one of the slants. Emulsify the growth on the slant by gently rubbing the bacterial film with the pipette, being careful not to tear the agar. Pipette the suspension back into dilution bottle “A.” Repeat this procedure a second time to remove any remaining growth on the agar slant, without disturbing the agar.

9.3.4 Make serial dilutions as follows:

9.3.4.1 Shake bottle “A” vigorously and pipette 1 mL to bottle “B”

9.3.4.2 Shake bottle “B” vigorously and pipette 1 mL to bottle “C”

9.3.4.3 Shake bottle “C” vigorously and pipette 1 mL to bottle “D”

9.3.4.4 Shake bottle “D” vigorously and pipette 10 mL to bottle “D2”; this should result in a final dilution of approximately 10 CFU / mL.

9.3.5 Filter 1- to 5-mL portions in triplicate from bottles “D” and “D2" according to the procedure in Section 10 to determine the number of CFU in the dilutions. The recommended target dilution and spike volume is one that produces 20 to 60 colonies per ADA-V plate. (It may be difficult to count plates with more than 60 colonies due to crowding.) Dilutions should be stored at 1EC to 5EC and may be used throughout the day they are prepared. However, it should be noted that the QC acceptance criteria were established using dilutions that were prepared immediately prior to spiking samples.

9.3.6 Analysts may practice the dilution scheme by placing filters on nutrient agar plates instead of ADA-V plates. After a growth pattern is determined and the analyst

can accurately determine the target concentrations, dilutions from Section 9.3.5 may be used for spiking IDC, ODC, and MS/MSD samples. However, multiple dilutions should be analyzed in replicate when new cultures are received from an outside source to ensure that the analyst can accurately spike target concentrations.

Note: If it is more convenient for your laboratory, an acceptable alternative to the dilution scheme presented in Section 9.3, is to pipette 11 mL of dilution D into a dilution bottle D2, which contains 99 mL of dilution water. There should be approximately 10^{10} Aeromonas hydrophila CFU per slant. Therefore, dilution bottles "A" through "D2" should contain approximately 10^{10}, 10^{8}, 10^{6}, 10^{4}, and 10^{3} CFU per dilution bottle, respectively. Depending on the growing conditions, these numbers may vary. As a result, until experience has been gained, more dilutions may need to be filtered to determine the appropriate dilution.

9.4 Initial demonstration of capability (IDC)—The IDC test is performed to demonstrate acceptable performance with the method prior to analysis of field samples. IDC tests must be accompanied by a dilution/rinse water blank(s) (Section 9.2.2), negative culture controls (Section 9.2.3), and media sterility checks (Section 9.2.6).

9.4.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Section 9.3.1 through 9.3.4.

9.4.2 For each of the four IDC test samples, spike enough volume of the appropriate dilution into 500 mL of sterile reagent water to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.4.3 Process IDC test samples according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. Submit 2 colonies per IDC test sample to the confirmation procedures in Section 10.11.

9.4.4 Using all four IDC sample results, compute the relative standard deviation (RSD) of Aeromonas CFU per 100 mL. (See glossary for definition of RSD.) Compare the RSD with the corresponding limits for IDC (Section 12). If the RSD meets the acceptance criteria, the system performance is acceptable and analysis of samples may begin. If the RSD falls outside the range, system performance is unacceptable. In this event, identify and correct the problem and repeat the test.

9.5 Dilution/rinse water blanks—On an ongoing basis, dilution/rinse water blanks must be processed at the beginning and end of each filtration series to check for possible cross-contamination. A filtration series ends when 30 minutes or more elapse between sample filtrations. An additional dilution/rinse water blank is also required for every 20 samples, if more than 20 samples are processed during a filtration series. For example, if a laboratory plans to run 30 samples during a filtration series, a dilution/rinse water blank should be processed at the beginning, middle, and end of the filtration series.

9.5.1 Process 100-mL dilution/rinse water blanks according to the procedures in Section 10, as appropriate.

9.5.2 No growth should appear in dilution/rinse water blanks. If growth appears, prepare new dilution/rinse water and reanalyze a 100-mL dilution/rinse water blank. If colonies are present after analyzing the new dilution/rinse water, assess laboratory technique and reagents. If growth in dilution/rinse water blank(s) is presumptively positive, all associated sample results should be discarded and sources re-sampled immediately.

9.6 Negative culture controls— Negative controls should be run whenever a new batch of medium or reagents is used. On an ongoing basis, the laboratory must perform, at a minimum, one negative culture control per week during weeks the laboratory analyzes field samples. Negative culture controls serve two purposes: to ensure the laboratories are familiar with the color and morphology of non-*Aeromonas* bacteria on ADA-V and to ensure that confirmation test results are appropriate. *E. coli* is (ATCC #25922) the negative culture control for oxidase, *Pseudomonas aeruginosa* (ATCC #27853) is the negative culture control for trehalose fermentation, and *Bacillus cereus* (ATCC #11778) is the negative culture control for indole.

9.6.1 Using pure cultures obtained from a qualified outside source (Sections 7.7 and 7.8), inoculate negative culture controls onto nutrient agar slants and incubate at 35°C ± 0.5°C for 24 ± 2 hours. Alternatively, nutrient agar slants may be inoculated up to 72 hours in advance. If nutrient agar slants will be incubated for more than 24 ± 2 hours, consider incubation at room temperature to ensure that the slants do not dry out prior to use.

9.6.2 For each negative culture control, place a membrane filter on an ADA-V plate, streak onto the filter, taking care not to break the filter, and incubate at 35°C ± 0.5°C for 24 ± 2 hours. Streaking on a filter will give the laboratory a more realistic example of the appearance of these organisms in field samples. Although not recommended, laboratories may streak directly onto the ADA-V (without the filter).

9.6.3 For each ADA-V negative culture control plate, pick a single colony, streak the colony onto a plate of nutrient agar medium (Section 7.9), and incubate at 35°C ± 0.5°C overnight to obtain isolated colonies. *Please note: Bacillus cereus typically grows only at the point of inoculation on ADA-V or not at all. If Bacillus cereus did not grow on the ADA-V plate, inoculate the streak plate from the nutrient agar slant that was originally used to inoculate the ADA-V plate.*

9.6.4 Negative culture control confirmation procedures

9.6.4.1 Oxidase negative culture control—From the streak plate, submit a single *E. coli* colony to the oxidase confirmation procedure described in Section 10.11.

9.6.4.2 Trehalose negative culture control—From the streak plate, submit a single *Pseudomonas aeruginosa* colony to the trehalose confirmation procedure described in Section 10.11.

9.6.4.3 Indole negative culture control—From the streak plate, submit a single *Bacillus cereus* colony to the indole confirmation procedure described in Section 10.11.

9.6.5 If any of the negative culture controls result in a positive confirmation, prepare, check and/or replace the associated media, reagents, and/or respective control organism and reanalyze the appropriate negative culture control(s). All presumptively positive colonies that have been archived from field samples (10 per sample) should be confirmed using media/reagents that exhibit the appropriate negative culture control response.

9.7 Matrix spike/matrix spike duplicate (MS/MSD)—The laboratory shall analyze MS/MSD samples when samples are first received from a finished drinking water source for which the laboratory has never before analyzed samples. Subsequently, 5% of field samples from a given source must include an MS/MSD test. Additional MS/MSD tests are also recommended when drinking water treatment is adjusted or when other events take place, for example, when scrubbing or replacing lines.

9.7.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Sections 9.3.1 through 9.3.4.

9.7.2 For each of the 500-mL MS and MSD test samples, spike enough volume of the appropriate dilution to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.7.3 Process MS/MSD test samples and an unspiked finished drinking water sample according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. (If the filter clogs during filtration, follow the instructions in Section 10, making sure to filter the same volume for both the MS and MSD. The same QC acceptance criteria apply.) Submit 10 colonies per IDC test sample to the confirmation procedures in Section 10.11.

Note: If results exceed the optimum range because of "background" target colonies (as indicated by the results of the unspiked matrix sample), the MS/MSD should be repeated and a smaller volume of sample, for example 200-mL, should be spiked.

9.7.4 For the MS and MSD test samples, calculate the number of confirmed Aeromonas CFU per 100 mL according to Section 11 and adjust based on any background Aeromonas observed in the unspiked sample.

9.7.5 Calculate the relative percent difference (RPD) using the following equation:

$$RPD = 100 \frac{| X_{MS} - X_{MSD} |}{X_{mean}}$$

where **RPD** is the relative percent difference
$\mathbf{X_{MS}}$ is the number of confirmed Aeromonas per 100 mL in the MS sample (minus the count of any background Aeromonas colonies observed in the unspiked finished water sample)
$\mathbf{X_{MSD}}$ is the number of confirmed Aeromonas per 100 mL in the MSD sample (minus the count of any background Aeromonas colonies observed in the unspiked finished water sample)
$\mathbf{X_{mean}}$ is the mean number of confirmed Aeromonas per 100 mL in the MS and MSD

9.7.6 Compare the RPD with the corresponding limits in Table 1 in Section 12. If the RPD meets the acceptance criteria, the system performance is acceptable and analysis of finished water samples from this source may continue. If the MS/MSD results are unacceptable and the ODC sample results associated with this batch of samples are acceptable, a matrix interference may be causing the poor results. If the MS/MSD results are unacceptable, all associated field data should be flagged.

9.8 Ongoing demonstration of capability (ODC)—The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate). The laboratory shall analyze one set of ODC samples after every 20 field and MS samples or one set per week that samples are analyzed, whichever occurs more frequently.

9.8.1 Prepare an Aeromonas QC spiking suspension according to the procedure in Section 9.3.1 through 9.3.4.

9.8.2 For each of the 500-mL positive control (PC) and positive control duplicate (PC/PCD) test samples, spike enough volume of the appropriate dilution into 500 mL of sterile reagent water to obtain the recommended target range of 20-60 CFU per filter. (It may be difficult to count plates with more than 60 colonies due to crowding.) Filter immediately after spiking.

9.8.3 Process PC/PCD test samples according to the procedure in Section 10.1 through 10.10 and record the number of presumptive positives for each sample. Submit 2 colonies per PC/PCD test sample to the confirmation procedures in Section 10.11.

9.8.4 Calculate the relative percent difference (RPD) using the following equation:

$$PRD = 100 \frac{| X - X_{PCD} |}{X_{mean}}$$

where
RPD is the relative percent difference
X_{PC} is the number of confirmed Aeromonas per 100 mL in the PC sample

X_{PCD} is the number of confirmed Aeromonas per 100 mL in the PCD sampleX_{mean} is the mean number of confirmed Aeromonas per 100 mL in the PC and PCD samples

9.8.5 Compare the RPD with the corresponding limits in Table 1 in Section 12. If the RPD meets the acceptance criteria, the system performance is acceptable and analysis of samples may continue. If RPD falls outside the range, system performance is unacceptable. Identify and correct the problem and perform another ODC test before continuing with the analysis of field samples.

9.8.6 As part of the QA program for the laboratory, method precision for ODC samples should be charted and records retained.

9.9 Analyst colony counting variability—If the laboratory has two or more analysts, each are required to count target colonies on the same membrane from one positive field sample per month. Compare each analyst's count of the target colonies. Counts should fall within 10% between analysts. If counts fail to fall within 10% of each other, analysts should perform additional sets of counts, until the number of target colonies counted fall within 10% between analysts for at least three consecutive samples. If there are no positive samples, an MS, MSD, or ODC sample can be used for this determination (MS or MSD are preferable to ODC samples, since they may have other background growth).

9.10 Autoclave sterilization verification—Verify autoclave sterilization monthly by placing Bacillus stearothermophilus spore suspensions or strips inside glassware. Sterilize at 121^{o}C for 15 minutes. Place in trypticase soy broth tubes and incubate at 55^{o}C for 48 hours. Check for growth to verify that sterilization was adequate. If sterilization was inadequate, determine appropriate time for autoclave sterilization. Filter sterilization may be used provided that these same QC steps are instituted for the filtrate.

10.0 Procedure

10.1 The membrane filter (MF) procedure with ampicillin-dextrin agar with vancomycin (ADA-V) is used to enumerate Aeromonas in finished waters.

10.2 Label each Petri dish with sample identification, preparation date, and analysis start date/time.

10.3 Use a sterile MF unit assembly (Section 6.18.3) at the beginning of each filtration series. The laboratory must sanitize each MF unit between filtrations by using a UV sanitizer, flowing steam, or boiling water for 2 min. A filtration series ends when 30 minutes or more elapse between sample filtrations.

10.4 Sterilize forceps with alcohol. Flame off excess alcohol. Using sterile forceps, place the MF (grid side up) over the sterilized funnel. Carefully place the top half of the filtration unit over the funnel and lock it in place.

10.5 Shake the sample bottle vigorously approximately 25 times to distribute the bacteria uniformly. Using aseptic technique, transfer one, 500-mL aliquot of sample to a single funnel. Use a graduated cylinder with a "to deliver" tolerance of approximately 2.5%.

Note: Laboratories must filter the entire 500-mL sample volume unless the filter clogs. If the filter clogs, a minimum of 100 mL of sample must be filtered, which may require multiple filtrations. If less than 500 mL are filtered and analyzed due to filter clogging, measure the residual, unfiltered volume to determine the volume filtered, and adjust the reporting limit accordingly.

10.6 Filter each sample under partial vacuum through a sterile membrane filter. Rinse the funnel after each sample filtration by filtering three, 30-mL portions of sterile buffered dilution water, being sure to thoroughly rinse the sides of the funnel.

10.7 Upon completion of the final rinse, disengage the vacuum and remove the funnel.

10.8 Using sterile forceps, immediately remove the MF and place it grid-side-up on the ADA-V medium with a rolling motion to avoid trapping air under the filter. Reseat the

membrane filter if bubbles occur. Place the inverted Petri dishes in the 35°C ± 0.5°C incubator within 30 minutes of preparation. Sterilize forceps and sanitize the MF unit between the analysis of each sample.

10.9 After 24 ± 2 hours of incubation at 35°C ± 0.5°C, count and record yellow colonies under magnification using a dissecting microscope.

10.10 Isolation of a yellow colony on ampicillin-dextrin agar with vancomycin (ADA-V) should be considered presumptively positive for Aeromonas.

10.11 Confirmation—All presumptive colonies, up to ten per sample, must be submitted to confirmation. In this method, any presumptive colony that is positive for oxidase (Section 10.11.2), ferments trehalose (Section 10.11.3), and produces indole (Section 10.11.4) is considered to be Aeromonas. If the result for any confirmation procedure is negative, no further confirmation steps are necessary. Slight variations in color and morphology may be present between different Aeromonas species grown on ADA-V medium. The colonies selected for confirmation should be representative of all yellow (presumptively positive) colony morphology types on ADA-V plate. For example, if 30 bright yellow colonies and 20 dull yellow colonies are observed, then 6 bright yellow and 4 dull yellow colonies should be submitted to confirmation.

Note: It is important to record the number of colonies of each presumptively positive morphological type so that the final density of Aeromonas can be reported based on percent confirmation of each morphological type. Also, the laboratory may submit more than ten presumptively positive colonies to the confirmation step.

10.11.1 Nutrient agar streak plate. To confirm as Aeromonas, pick a colony and streak the colony onto a plate of nutrient agar medium (Section 7.9) and incubate at 35°C ± 0.5°C overnight to obtain isolated colonies.

10.11.2 Oxidase confirmation. Apply a very small amount of a discreet colony from the nutrient agar to the oxidase dry slide using a wooden or plastic applicator. Do not use iron or other reactive wire because it may cause false positive reactions. Also, do not transfer any medium with the culture material, as this could lead to inconsistent results. A blue/purple color reaction within 10 seconds is considered a positive oxidase test. For commercially-prepared reagent, adhere to manufacturer's expiration date. Freshly-made solutions should be used within one week. Please note: This method was validated using nutrient agar, if the oxidase reagent is to be dropped directly on colonies, use tryptic soy agar plates because nutrient agar plates give inconsistent results. The use of tryptic soy agar plates for streaking (Section 10.11.1) has not been validated and is considered a method modification and, as a result, the laboratory must demonstrate acceptable performance for the QC analyses described in Section 9.2.12.

Note: Timing of the color reaction is critical, as some Gram-positive bacteria may give false positives after 10 seconds. Also, it is important to put just a small amount of the colony on the oxidase dry slide or saturated pad, as too much bacteria can also cause a false positive oxidase test.

10.11.3 Trehalose confirmation. If the oxidase test is positive, then test for trehalose fermentation. Trehalose fermentation is determined by inoculating a tube containing 3-10 mL (depending on the size of the tube used - fill about half full) of 0.5% trehalose in purple broth base (Section 7.11) with a colony from the nutrient agar and incubating at 35°C ± 0.5°C for 24 ± 2 hours. A change in color of the medium from purple to yellow is considered a positive for trehalose fermentation.

10.11.4 Indole confirmation. If the oxidase and trehalose tests are positive, then test for indole production. (If the laboratory prefers, the indole confirmation procedure may be started on the same day as the trehalose confirmation.) Indole production is determined by inoculating a tube containing 3-10 mL (depending on the size of the tube used - fill about half full) of tryptone broth (Section 7.12) with a colony from the nutrient agar and incubating at 35°C ± 0.5°C for 24 ± 2 hours. After incubation, add 0.2 to 0.3 mL (4 to 6 drops) of Kovac's test reagent (Section 7.13) to each tube, let stand for approximately 10 minutes and observe results. A pink to red color in the surface layer constitutes a positive indole test. The original color of the Kovac's reagent indicates a negative indole test. An

orange color probably indicates the presence of skatole, a breakdown product of indole, and is considered a positive result.
10.11.5 If a colony is oxidase, trehalose, and indole positive, report as a confirmed Aeromonas and archive the colony for further identification.
Note: If samples are to be archived for further analysis to determine species or hybridization group, from the nutrient agar plate (Section 10.11.1), inoculate a nutrient agar slant for short term use or shipment to another laboratory.

11.0 Data Analysis and Calculations
11.1 See Standard Methods for the Examination of Water and Wastewater (Reference 15.2) for general counting rules. The density of Aeromonas determined by the membrane filter (MF) procedure is calculated by direct identification and enumeration of yellow colonies by a dissecting microscope (Section 6.19) followed by oxidase, trehalose, and indole confirmation. Bacterial density is recorded as presumptive Aeromonas colony forming units (CFU) per 100 mL of sample and confirmed Aeromonas CFU per 100 mL.
11.2 Counting colonies on ADA-V
11.2.1 Record the number of presumptive Aeromonas CFU/100mL. If there is more than one morphological type that is considered to be presumptively positive, record the number of presumptive positives for each morphological type, as well as the total number of presumptive positives.
11.2.2 If there are more than 200 colonies, including background colonies, report results as too numerous to count (TNTC) and resample. If the filter is TNTC with more background colonies than presumptive aeromonads, split the 500 mL resample between 3 or 4 filters in order to better differentiate the colony morphology types. If the filter is TNTC with mostly aeromonads, a minimum of three dilutions (e.g. 100 mL, 10 mL and 1 mL) should be analyzed.
11.2.3 If the colonies are not discrete and appear to be growing together, report results as confluent growth (CG) and resample.

11.3 Confirmation and calculation of Aeromonas density
11.3.1 In this method, any presumptive colony that is positive for oxidase (Section 10.11.2), ferments trehalose (Section 10.11.3), and produces indole (Section 10.11.4) is considered to be Aeromonas. For the final density of confirmed Aeromonas, adjust the initial, presumptive count based on the positive confirmation percentage for each presumptively positive morphological type and report as confirmed CFU per 100 mL.
11.3.2 Calculate the number of positive confirmations for each presumptively positive morphological type from all filters of a given sample using the following equation:

$$\left(\frac{\text{Number positively confirmed}}{\text{Number submitted to confirmation}} \text{ X Number of presumptive positives}\right) = A$$

$$A \text{ X } \frac{100}{\text{mL filtered}} = \text{Confirmed Aeromonas /100 mL}$$

11.3.3 Record the number of confirmed Aeromonas per 100 mL for each colony morphology.
11.3.4 Sum the number of confirmed Aeromonas per 100 mL for all presumptively positive colony types (Section 11.3.2) and report as the density of confirmed Aeromonas per 100 mL.
11.3.5 Example 1: In this example, 500 mL of sample was filtered and two different morphological types of presumptively positive colonies were observed.

Example 1 Morphological Description	Number of presumptively positive colonies per volume filtered	Number submitted to confirmation steps	Number positively confirmed	Number of confirmed *Aeromonas* per 100 mL
Type A: Bright yellow, round, opaque	30	6	6	6
Type B: Dull yellow, oval, translucent	20	4	3	3
			Total number of confirmed *Aeromonas* per sample:	**9 per 100 mL**

Example 1 results in 9 confirmed *Aeromonas* / 100 mL.
11.3.6 Example 2: In this example, ***200 mL*** of sample was filtered and two different morphological types of presumptively positive colonies were observed.

Example 2 Morphological Description	Number of presumptively positive colonies per volume filtered	Number submitted to confirmation steps	Number positively confirmed	Number of confirmed *Aeromonas* per 100 mL
Type A: Dull yellow, round, opaque	40	5	5	20
Type B: Dull yellow, round, translucent	40	5	3	12
			Total number of confirmed *Aeromonas* per sample:	**32 per 100 mL**

Example 2 results in 32 confirmed *Aeromonas* / 100 mL.
11.3.7 If there were no presumptively positive colonies or if none of the presumptive colonies are confirmed, then report the results as less than the detection limit (DL) in CFU per 100 mL based on sample volume filtered. If less than 500 mL are filtered, then adjust the reporting limit per 100 mL accordingly. The DL may be calculated as follows:

DL per 100 mL = 100 / volume filtered CFU per 100mL

11.3.7.1 Example 3: If 500 mL of sample was filtered and there were no confirmed colonies, then report as <0.2 CFU/100 mL.
11.3.7.2 Example 4: If 100 mL of sample was filtered and there were no confirmed colonies, then report as <1.0 CFU/100 mL.

12.0 Method Performance
12.1 Specificity of media **12.1.1** Please refer to Section 16, Table 2, for results of *Aeromonas* growth after 24 hours on ADA at 30EC and 35EC and ADA-V at 35EC .
12.1.2 ADA-V was able to support the growth of the *Aeromonas* species (*hydrophila, caviae,* and *veronii/sobria*) most often associated with human disease.
12.1.3 Efforts continue to identify colonies which give a presumptive positive on the ADA-V media but do not confirm.
12.2 The QC acceptance criteria listed in Table 1, below are based on data generated through the interlaboratory validation of Method 1605 involving 11 laboratories and 11 finished drinking water matrices. Detailed method QC procedures applicable to these criteria are discussed in Section 9.

Table 1. QC Acceptance Criteria for Method 1605 QC

QC specification	**Maximum acceptable precision**
Initial demonstration of capability (IDC): This test will require the analysis of 4 spiked reagent water samples	RSD = 22%
Ongoing demonstration of capability (ODC): This test will require the analysis of 2 spiked reagent water samples	RPD = 37%
Matrix spike/matrix spike duplicate (MS/MSD) precision: This test will require the analysis of 2 spiked finished water (matrix) samples	RPD = 48%

13.0 Pollution Prevention
13.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.
13.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.
14.0 Waste Management
14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the biohazard waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

14.2 Samples, reference materials, and equipment known or suspected of having bacterial contamination from this work must be sterilized prior to disposal.
14.3 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less is Better: Laboratory Chemical Management for Waste Reduction," both of which are available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.
15.0 References
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15.5 Manual for the Certification of Laboratories Analyzing Drinking Water. 1997. 4th Edition. EPA-815-B-97-001. Office of Ground Water and Drinking Water. U.S. EPA.
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15.7 Reagent Chemicals, American Chemical Society Specifications. American Chemical Society, Washington, D.C.
15.8 Handfield, M., P. Simard, and R. Letarte. 1996. Differential media for quantitative recovery of waterborne *Aeromonas hydrophila*. Applied Environmental Microbiology 62:3544-3547.
15.9 Standard Methods for the Examination of Water and Wastewater. 1995. 19th Edition. Eds. A.D. Eaton, L.S. Clesceri, and A. Greenberg. American Public Health Association, American Water Works Association, and Water Environment Federation. American Public Health Association, Washington, D.C., publisher.
15.10 Janda, J.M. and S.L. Abbott. 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. Journal of Clinical Infectious diseases. 27:332-344.

16.0 Tables and Flowcharts

Collection #	Hybridization group	*Aeromonas* species	ADA at 30oC	ADA at 35oC	ADA-V at 35oC
ATCC 7966	Group 1	*hydrophila*	+	+	+
ATCC 35654	Group 1	*hydrophila*	+	+	+
AMC 12723-W	Group 1	*hydrophila*	+	+	+
ATCC 51108	Group 2	*bestiarum*	+	+	+
AMC 14228-V	Group 2	*bestiarum*	+	+	+
ATCC 336581	Group 3	*salmonicida/salmonicida*	-	-	NA
AMC 15228-V	Group 3	*salmonicida*	+	+	+
ATCC 15468	Group 4	*caviae*	+	+	+
MML 1685-E	Group 4	*caviae*	+	+	+
ATCC 33907	Group 5	*media*	-	-	NA
AMC Leftwich	Group 5	*media*	-	-	NA
ATCC 233091	Group 6	*eucrenophila*	+	-	NA
ATCC 35993	Group 7	*sobria*	+	+	+
Muldoon SMHC	Group 7	*sobria*	+	+	+
ATCC 9071	Group 8	*veronii/sobria*	+	+	+
AMC 1123-W	Group 8	*veronii/sobria*	+	+	+
ATCC 43700	Group 12	*schubertii*	+2	+5	+5
AMC 1108-W	Group 12	*schubertii*	+	-	NA
ATCC 496573	unknown	*trota*	-	-	NA
NMRI 206	unknown	*trota*	-	-	NA
ATCC 51208	unknown	*allosaccharophila*	+	+	+
ATCC 49568	Group 9	*jandaei*	+	+	+
AS 14	Group 9	*jandaei*	+	+	+
ATCC 35622	Group 10	*veronii/veronii*	+	+	+
WR 4659	Group 10	*veronii/veronii*	+	+	+
CECT 4342	Group 11	*encheleia*	+	-	NA
LMG 175414	unknown	*popoffii*	+	+	+
AMC (ATCC 35941)	unknown	ornithine positive	-	-	NA
AMC (ATCC 43946)	unknown	Group 501	+	+	+

CDC 0434-84 Group 3 Motile Group 3 + + +
(1) Respective *Aeromonas* cultures grew on ADA medium when streaked, but not when filtered.

(2) Respective Aeromonas cultures grew when streaked on ADA medium at 30°C, however filtration was not performed with these cultures.(3) Respective Aeromonas cultures did not grow on ADA medium when streaked.(4) Respective Aeromonas cultures grew poorly on ADA medium at both temperatures and on ADA-V at 35°C. The same pattern of poor growth was also observed on non-selective media.(5) Respective Aeromonas cultures grew poorly on ADA and ADA-V medium at 35°C. The same pattern of poor growth was also observed on non-selective media. Results: A Based on ADA results, it was assumed that the culture would not grow on ADA-V at 35°C.
+ positive growth
-No growth

ATCC = American Type Culture Collection, Manassas, VA Other cultures were obtained from Amy Carnahan, University of Maryland. Serial dilutions representing approximately 10-200 CFU were filtered and the membrane placed on ADA or ADA-V medium as described in Section 10. Additional membranes representing the same dilution for each of the respective cultures were placed on brain heart infusion agar as a control.

17.3 Definitions
Confirmed colonies—Presumptively positive colonies that test positive for oxidase, ferment trehalose, and produce indole

Dilution/rinse water blank—A 100-mL aliquot of dilution/rinse water that is treated exactly as a sample and carried through all portions of the procedure until determined to be negative or positive. The Dilution/rinse water blank is used to determine if the sample has become contaminated by the introduction of a foreign microorganism through poor technique.

Initial demonstration of capability (IDC)—The IDC test is performed to demonstrate acceptable performance with the method prior to analysis of field samples.

Must—This action, activity, or procedural step is required.

Negative culture control—A non-*Aeromonas* bacteria processed to ensure the laboratories are familiar with the color and morphology of non-*Aeromonas* bacteria on ADA-V and to ensure that confirmation test results are appropriate.

Ongoing demonstration of capability (ODC)—The laboratory shall demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples (positive control/positive control duplicate).

Positive control—A 500-mL reagent water spiked with 20 - 80 CFU of Aeromonas. The positive control is analyzed exactly like a sample. Its purpose is to ensure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

Presumptive positive colonies—Colonies that are yellow on ADA-V.

Relative Standard Deviation (RSD)—The standard deviation times 100 divided by the mean.

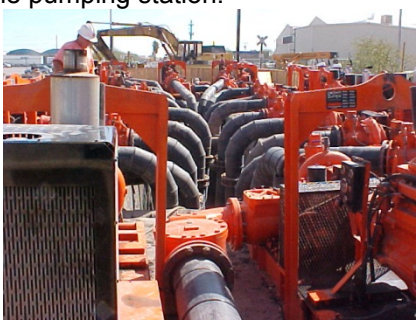
Selective medium—A culture medium designed to suppress the growth of unwanted microorganisms and encourage the growth of the target bacteria.

Should—This action, activity, or procedural step is suggested but not required.

Pumps and Lift Stations Section

Lift Station: A facility in a sewer system consisting of a receiving chamber, pumping equipment and associated drive and control devices which collect and lift wastewater to a higher elevation when the continuance of the sewer at reasonable slopes would involve excessive trench depths; or that collects and raises wastewater through the use of force mains from areas too low to drain into available sewers. There should not be an odor coming from a Lift Station.

Pumping Station: A relatively large sewage pumping installation designed not only to lift sewage to a higher elevation, but also to convey it through force mains to gravity flow points located relatively long distances from the pumping station.



Pumps at a temporary sewer manhole by-pass.

Lift Stations

Sewer pipes are generally gravity driven. Wastewater flows slowly downhill until it reaches a certain low point. Then, pumps or "lift" stations push the wastewater back uphill to a high point where gravity can once again take over the process.

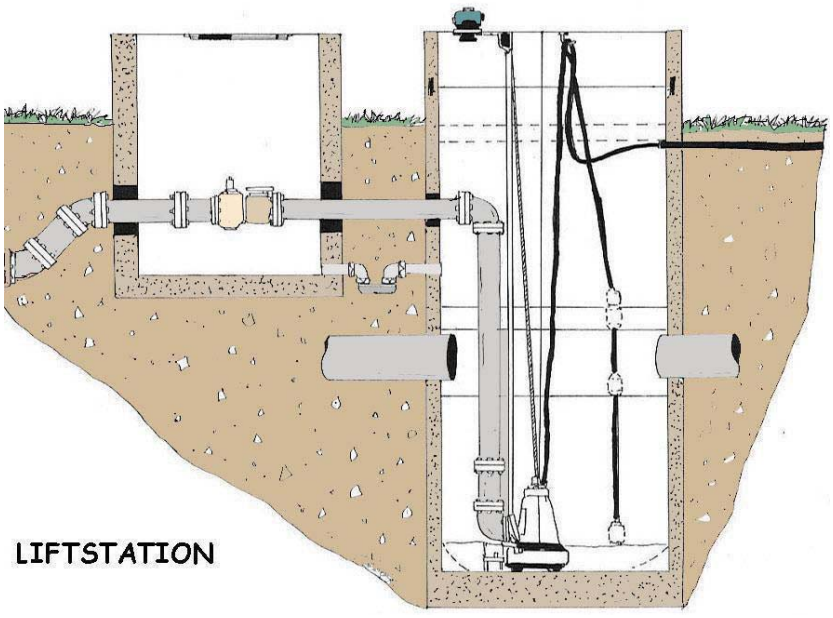
Lift stations are used in sanitary sewer systems where water is accumulated in wet wells and then pumped to a higher elevation. They are generally designed to operate continuously to keep sewerage from backing up through the system. That means that most lift stations have a backup electrical supply in the event that normal power is disrupted.

Most wastewater collection systems will have installed radio telemetry, or SCADA systems. The telemetry system is used to monitor and control pump stations via computer at the WW Collections facility.

This system gives up to the minute pump station status such as wet well level, pump performance, electrical power conditions, etc. This allows our technicians to prevent wastewater spills and protect public health. Using telemetry, we have the ability to identify potential problems instantaneously and take the proper steps to rectify the situation before it becomes a public health risk.

A Lift Station contains 4 main Components:

- A wet well - usually 15+ ft. in depth and 8ft. in diameter - that houses two submersible pumps (there are some stations with up to 5 submersibles) of varying horsepower, discharging piping and floats that operate the pumps and keep a set level in the well.
- A dry well that houses the piping and valves that prevent backflow in the station, and can lock connection used to bypass the submersibles in an emergency situation.
- An electrical panel houses control for the submersible pumps. It also houses the telemetry used to monitor and control the station remotely.
- A “Log Book” or “Station Book” which contains the records and maps of the Lift Station’s area.



Collection Systems O&M Section

Sewer Cleaning and Inspection
As sewer system networks age, the risk of deterioration, blockages, and collapses becomes a major concern. As a result, municipalities worldwide are taking proactive measures to improve performance levels of their sewer systems.

Cleaning and inspecting sewer lines are essential to maintaining a properly functioning system; these activities further a community's reinvestment into its wastewater infrastructure.

Inspection Techniques
Inspection programs are required to determine current sewer conditions and to aid in planning a maintenance strategy. Ideally, sewer line inspections need to take place during low flow conditions. If the flow conditions can potentially overtop the camera, then the inspection should be performed during low flow times between midnight and 5 AM, or the sewer lines can be temporarily plugged to reduce the flow. Most sewer lines are inspected using one or more of the following techniques:
• Closed-circuit television (CCTV).
• Cameras.
• Visual inspection.
• Lamping inspection.

Television (TV) inspections are the most frequently used, most cost efficient in the long term, and most effective method to inspect the internal condition of a sewer. CCTV inspections are recommended for sewer lines with diameters of 0.1-1.2 m (4 - 48 inches.) The CCTV camera must be assembled to keep the lens as close as possible to the center of the pipe. In larger sewers, the camera and lights are attached to a raft, which is floated through the sewer from one manhole to the next. To see details of the sewer walls, the camera and lights swivel both vertically and horizontally.

In smaller sewers, the cable and camera are attached to a sled, to which a parachute or droge is attached and floated from one manhole to the next. Documentation of inspections is very critical to a successful operation and maintenance (O&M) program. CCTV inspections produce a video record of the inspection that can be used for future reference. In larger sewers where the surface access points are more than 300 m (1000 linear feet) apart, camera inspections are commonly performed. This technique involves a raft-mounted film camera and strobe light. This method requires less power than the CCTV, so the power cable is smaller and more manageable. Inspections using a camera are documented on Polaroid still photographs that are referenced in a log book according to date, time, and location.

Visual inspections are vital in fully understanding the condition of a sewer system. Visual inspections of manholes and pipelines are comprised of surface and internal inspections. Operators should pay specific attention to sunken areas in the groundcover above a sewer line and areas with ponding water. In addition, inspectors should thoroughly check the physical conditions of stream crossings, the conditions of manhole frames and covers or any exposed brickwork, and the visibility of manholes and other structures. For large sewer lines, a walk-through or internal inspection is recommended. This inspection requires the operator to enter a manhole, the channel, and the pipeline, and assess the condition of the manhole frame, cover, and chimney, and the sewer walls above the flow line.

When entering a manhole or sewer line, it is very important to observe the latest Occupational Safety and Health Administration confined space regulations. If entering the manhole is not feasible, mirrors can be used. Mirrors are usually placed at two adjacent manholes to reflect the interior of the sewer line. Lamping inspections are commonly used in low priority pipes, which tend to be pipes that are less than 20 years old.

Lamping is also commonly used on projects where funds are extremely limited. In the lamping technique, a camera is inserted and lowered into a maintenance hole and then positioned at the center of the junction of a manhole frame and the sewer. Visual images of the pipe interior are then recorded with the camera. Several specialized inspection techniques have been recently developed worldwide. Light-line based and sonar-based equipment that measures the internal cross-sectional profile of sewer systems.

Sonar technology could be very useful in inspecting depressed sewers (inverted siphons), where the pipe is continually full of water under pressure. Melbourne Water and CSIRO Division of Manufacturing Technology have introduced a new technology called PIRAT, which consists of an in-pipe vehicle with a laser scanner. This instrument is capable of making a quantitative and automatic assessment of sewer conditions. The geometric data that is gathered is then used to recognize, identify, and rate defects found in the sewer lines.

Cleaning Techniques

To maintain its proper function, a sewer system needs a cleaning schedule. There are several traditional cleaning techniques used to clear blockages and to act as preventative maintenance tools. When cleaning sewer lines, local communities need to be aware of EPA regulations on solid and hazardous waste as defined in 40 CFR 261. In order to comply with state guidelines on testing and disposal of hazardous waste, check with the local authorities.

Hydraulic cleaning developments have also been emerging on the international frontier. France and Germany have developed several innovative flushing systems using a ‘dam break’ concept. France has developed a flushing system called the Hydrass. The design of the Hydrass consists of a gate that pivots on a hinge to a near horizontal position. As the gate opens and releases a flow, a flush wave is generated that subsequently washes out any deposited sediments. Germany has also developed a similar system called GNA Hydroself®. This is a flushing system that requires no electricity, no maintenance and no fresh water. The Hydroself® consists of a hydraulically-operated gate and a concrete wall section constructed to store the flush water. This system can be installed into a large diameter sewer.

There appears to be no limit on the flushing length, as more flush water may be stored without incurring any additional construction or operating costs. Another example of such a technology is seen in the Brussels Sewer System. A wagon with a flushing vane physically moves along the sewer and disturbs the sediments so that they are transported with the sewer flow.

Although all of these methods have proven effective in maintaining sewer systems, the ideal method of reducing and controlling the materials found in sewer lines is education and pollution prevention. The public needs to be informed that common household substances such as grease and oil need to be disposed in the garbage in closed containers, and not into the sewer lines. This approach will not only minimize a homeowner’s plumbing problems, but will also help keep the sewer lines clear.

In recent years, new methodologies and accelerated programs have been developed to take advantage of the information obtained from sewer line maintenance operations. Such programs incorporate information gathered from various maintenance activities with basic sewer evaluations to create a system that can remedy and prevent future malfunctions and failures more effectively and efficiently. Some cities have attempted to establish a program that would optimize existing maintenance activities to reduce customer complaints, sanitary sewer overflows, time and money spent on sewer blockages, and other reactive maintenance activities. Their plan is based on maintenance frequencies, system performance, and maintenance costs over a period of time. This plan was developed using Geographical Information System (GIS) and historical data to show areas of complaints, back-ups, and general maintenance information for the area.

Technology Uses and Applications

Mechanical
Rodding
• Uses an engine and a drive unit with continuous rods or sectional rods.
• As blades rotate they break up grease deposits, cut roots, and loosen debris.
• Rodders also help thread the cables used for TV inspections and bucket machines.
• Most effective in lines up to 12 inches in diameter.

Bucket Machine
• Cylindrical device, closed on one end with 2 opposing hinged jaws at the other.
• Jaws open and scrape off the material and deposit it in the bucket.
• Partially removes large deposits of silt, sand, gravel, and some types of solid waste.

Hydraulic
Balling
• A threaded rubber cleaning ball that spins and scrubs the pipe interior as flow increases in the sewer line.
• Removes deposits of settled inorganic material and grease build-up.
• Most effective in sewers ranging in size from 5-24 inches.

Flushing
• Introduces a heavy flow of water into the line at a manhole.
• Removes floatables and some sand and grit.
• Most effective when used in combination with other mechanical operations, such as rodding or bucket machine cleaning.

Jetting
• Directs high velocities of water against pipe walls.
• Removes debris and grease build-up, clears blockages, and cuts roots within small diameter pipes.
• Efficient for routine cleaning of small diameter, low flow sewers.

Technology Applications

Scooter
• Round, rubber-rimmed, hinged metal shield that is mounted on a steel framework on small wheels. The shield works as a plug to build a head of water.
• Scours the inner walls of the pipe lines.
• Effective in removing heavy debris and cleaning grease from line.

Kites, Bags, and Poly Pigs
• Similar in function to the ball.
• Rigid rims on bag and kite induce a scouring action.
• Effective in moving accumulations of decayed debris and grease downstream.

Silt Traps
• Collect sediments at convenient locations.
• Must be emptied on a regular basis as part of the maintenance program.

Grease Traps and Sand/Oil Interceptors
• The ultimate solution to grease build-up is to trap and remove it.
• These devices are required by some uniform building codes and/or sewer-use ordinances. Typically sand/oil interceptors are required for automotive business discharge.
• Need to be thoroughly cleaned to function properly.

• Cleaning frequency varies from twice a month to once every 6 months, depending on the amount of grease in the discharge.
• Need to educate restaurant and automobile businesses about the need to maintain these traps.

Chemicals
Before using these chemicals review the Material Safety Data Sheets (MSDS) and consult the local authorities on the proper use of chemicals as per local ordinance and the proper disposal of the chemicals used in the operation. If assistance or guidance is needed regarding the application of certain chemicals, contact the U.S. EPA or state water pollution control agency.

• Used to control roots, grease, odors (H2S gas), concrete corrosion, rodents and insects.
• *Root Control* - longer lasting effects than power rodder (approximately 2-5 years).
• *H2S gas* - some common chemicals used are chlorine (Cl2), hydrogen peroxide (H2O2), pure oxygen (O2), air, lime (Ca(OH2)), sodium hydroxide (NaOH), and iron salts.
• *Grease and soap problems* - some common chemicals used are bioacids, digester, enzymes, bacteria cultures, catalysts, caustics, hydroxides, and neutralizers.
Source: Information provided by Arbour and Kerri, 1997 and Sharon, 1989.

Most cities that take advantage of this are able to determine that as the maintenance frequency increased, there was an increase in system performance. Garland recommended 70 inspections and maintenance activities for every 30 cleanings. Inspections are considered more important because they help define and prevent future problems.

A study performed by the American Society of Civil Engineers reports that the most important maintenance activities are cleaning and CCTV inspections. A maintenance plan attempts to develop a strategy and priority for maintaining pipes based on several of the following factors:
• Problems- frequency and location; 80 percent of problems occur in 25 percent of the system (Hardin and Messer, 1997).
• Age- older systems have a greater risk of deterioration than newly constructed sewers.
• Construction material- pipes constructed of materials that are susceptible to corrosion have a greater potential of deterioration and potential collapse. Non-reinforced concrete pipes, brick pipes, and asbestos cement pipes are examples of pipes susceptible to corrosion.
• Pipe diameter/volume conveyed- pipes that carry larger volumes take precedence over pipes that carry a smaller volume.
• Location- pipes located on shallow slopes or in flood prone areas have a higher priority.
• Force main vs. gravity-force mains have a higher priority than gravity, size for size, due to the complexity of the cleaning and repairs.
• Subsurface conditions- depth to groundwater, depth to bedrock, soil properties (classification, strength, porosity, compressibility, frost susceptibility, erodibility, and pH).
• Corrosion potential- Hydrogen Sulfide (H2S) is responsible for corroding sewers, structures, and equipment used in wastewater collection systems. The interior conditions of the pipes need to be monitored and treatment needs to be implemented to prevent the growth of slime bacteria and the production of H2S gases.

Activity Average (% of system/year)
Cleaning 29.9
Root removal 2.9
Manhole inspection 19.8
CCTV inspection 6.8
Smoke testing 7.8
Source: ASCE, 1998.

Advantages and Disadvantages

The primary benefit of implementing a sewer maintenance program is the reduction of SSOs, basement backups, and other releases of wastewater from the collection system due to substandard sewer conditions. Improper handling of instruments and chemicals used in inspecting and maintaining sewer lines may cause environmental harm.

Examples include:
• Improperly disposing of collected materials and chemicals from cleaning operations.
• Improperly handling chemical powdered dyes.
• Inadequately maintaining inspection devices.

Visual Inspection
In smaller sewers, the scope of problems detected is minimal because the only portion of the sewer that can be seen in detail in near the manhole. Therefore, any definitive information on cracks or other structural problems is unlikely. However, this method does provide information needed to make decisions on rehabilitation.

Camera Inspection
When performing a camera inspection in a large diameter sewer, the inspection crew is essentially taking photographs haphazardly, and as a result, the photographs tend to be less comprehensive.

Closed Circuit Television (CCTV)
This method requires late night inspection and as a result the TV operators are vulnerable to lapses in concentration. CCTV inspections are also expensive and time consuming. The video camera does not fit into the pipe and during the inspection it remains only in the maintenance hole.

Lamping Inspection
As a result, only the first 10 feet of the pipe can be viewed or inspected using this method.
Source: Water Pollution Control Federation, 1989. Some instruments have a tendency to become coated with petroleum based residues and if not handled properly they can become a fire hazard.

Cleaning Method Limitations

Balling, Jetting, Scooter: In general, these methods are only successful when necessary water pressure or head is maintained without flooding basements or houses at low elevations. Jetting - The main limitation of this technique is that cautions need to be used in areas with basement fixtures and in steep-grade hill areas.

Balling - Balling cannot be used effectively in pipes with bad offset joints or protruding service connections because the ball can become distorted.

Scooter - When cleaning larger lines, the manholes need to be designed to a larger size in order to receive and retrieve the equipment. Otherwise, the scooter needs to be assembled in the manhole. Caution also needs to be used in areas with basement fixtures and in steep-grade hill areas.

Bucket Machine
This device has been known to damage sewers. The bucket machine cannot be used when the line is completely plugged because this prevents the cable from being threaded from one manhole to the next. Set-up of this equipment is time-consuming.

Flushing This method is not very effective in removing heavy solids. Flushing does not remedy this problem because it only achieves temporary movement of debris from one section to another in the system.

High Velocity Cleaner
The efficiency and effectiveness of removing debris by this method decreases as the cross-sectional areas of the pipe increase. Backups into residences have been known to occur when this method has been used by inexperienced operators. Even experienced operators require extra time to clear pipes of roots and grease.

Kite or Bag When using this method, use caution in locations with basement fixtures and steep-grade hill areas.

Rodding Continuous rods are harder to retrieve and repair if broken and they are not useful in lines with a diameter of greater than 300 mm (0.984 feet) because the rods have a tendency to coil and bend. This device also does not effectively remove sand or grit, but may only loosen the material to be flushed out at a later time. Source: U.S. EPA, 1993.

Limitations of Cleaning Methods
• Sewer Cleaning and Stoppage Section- this section responds to customer complaints, pinpoints problems within the lines, and clears all blockages.
• TV Section- this section locates defects and building sewer connections (also referred to as taps) within the system.
• Preventive Maintenance Section- this section cleans and inspects the lines and also provides for Quality Assurance and Quality Control (QA/QC).

Most of collection inspections use CCTV system. However, a large percent of the lines in the worst and oldest sections of the system are inspected visually. Visual inspections are also used in the most recently installed lines and manholes. The collection system will normally utilize a variety of cleaning methods including jetting, high velocity cleaning, rodding, bucket machining, and using stop trucks (sectional rods with an attached motor).

As part of a preventive maintenance approach, most collection system operators also have been using combination trucks with both flush and vacuum systems. To control roots, most collection system operators uses a vapor rooter eradication system which can ensure that no roots return to the line for up to five years. The cleaning and inspection crews will usually consist of two members to operate each of the combination trucks and TV trucks.

Collection Systems, Lift Stations

Wastewater lift stations are facilities designed to move wastewater from lower to higher elevation through pipes. Key elements of lift stations include a wastewater receiving well (wet-well), often equipped with a screen or grinding to remove coarse materials; pumps and piping with associated valves; motors; a power supply system; an equipment control and alarm system; and an odor control system and ventilation system.

Lift station equipment and systems are often installed in an enclosed structure. They can be constructed on-site (custom-designed) or prefabricated. Lift station capacities range from 20 gallons per minute to more than 100,000 gallons per minute. Pre-fabricated lift stations generally have capacities of up to 10,000 gallons per minute.

Centrifugal pumps are commonly used in lift stations. A trapped air column, or bubbler system, that senses pressure and level is commonly used for pump station control. Other control alternatives include electrodes placed at cut-off levels, floats, mechanical clutches, and floating mercury switches. A more sophisticated control operation involves the use of variable speed drives. Lift stations are typically provided with equipment for easy pump removal. Floor access hatches or openings above the pump room and an overhead monorail beam, bridge crane, or portable hoist are commonly used.

The two most common types of lift stations are the dry-pit or dry-well and submersible lift stations. In dry-well lift stations, pumps and valves are housed in a pump room (dry pit or dry-well), that is easily accessible. The wet-well is a separate chamber attached or located adjacent to the dry-well (pump room) structure.

Submersible lift stations do not have a separate pump room; the lift station header piping, associated valves, and flow meters are located in a separate dry vault at grade for easy access. Submersible lift stations include sealed pumps that operate submerged in the wet-well. These are removed to the surface periodically and reinstalled using guide rails and a hoist. A key advantage of dry-well lift stations is that they allow easy access for routine visual inspection and maintenance. In general, they are easier to repair than submersible pumps. An advantage of submersible lift stations is that they typically cost less than dry-well stations and operate without frequent pump maintenance.

Submersible lift stations do not usually include large aboveground structures and tend to blend in with their surrounding environment in residential areas. They require less space and are easier and less expensive to construct for wastewater flow capacities of 10,000gallons per minute or less.

Applicability
Lift stations are used to move wastewater from lower to higher elevation, particularly where the elevation of the source is not sufficient for gravity flow and/or when the use of gravity conveyance will result in excessive excavation depths and high sewer construction costs.

Current Status
Lift stations are widely used in wastewater conveyance systems. Dry-well lift stations have been used in the industry for many years. However, the current industry-wide trend is to replace drywell lift stations of small and medium size (typically less than 6,350 gallons per minute with submersible lift stations mainly because of lower costs, a smaller footprint, and simplified operation and maintenance. Variable speed pumping is often used to optimize pump performance and minimize power use. Several types of variable-speed pumping equipment are available, including variable voltage and frequency drives, eddy current couplings, and mechanical variable-speed drives.

Variable-speed pumping can reduce the size and cost of the wetwell and allows the pumps to operate at maximum efficiency under a variety of flow conditions. Because variable-speed pumping allows lift station discharge to match inflow, only nominal wet-well storage volume is required and the well water level is maintained at a near constant elevation. Variable-speed pumping may allow a given flow range to be achieved with fewer pumps than a constant-speed alternative.

Variable-speed stations also minimize the number of pump starts and stops, reducing mechanical wear. Although there is significant energy saving potential for stations with large friction losses, it may not justify the additional capital costs unless the cost of power is relatively high. Variable speed equipment also requires more room within the lift station and may produce more noise and heat than constant speed pumps.

Lift stations are complex facilities with many auxiliary systems. Therefore, they are less reliable than gravity wastewater conveyance. However, lift station reliability can be significantly improved by providing stand-by equipment (pumps and controls) and emergency power supply systems. In addition, lift station reliability is improved by using non-clog pumps suitable for the particular wastewater quality and by applying emergency alarm and automatic control systems.

Advantages
Lift stations are used to reduce the capital cost of sewer system construction. When gravity sewers are installed in trenches deeper than 10 feet, the cost of sewer line installation increases significantly because of the more complex and costly excavation equipment and trench shoring techniques required. The size of the gravity sewer lines is dependent on the minimum pipe slope and flow. Pumping wastewater can convey the same flow using smaller pipeline size at shallower depth, and thereby, reducing pipeline costs.

Disadvantages
Compared to sewer lines where gravity drives wastewater flow, lift stations require a source of electric power. If the power supply is interrupted, flow conveyance is discontinued and can result in flooding upstream of the lift station, It can also interrupt the normal operation of the downstream wastewater conveyance and treatment facilities. This limitation is typically addressed by providing an emergency power supply.

Key disadvantages of lift stations include the high cost to construct and maintain and the potential for odors and noise. Lift stations also require a significant amount of power, are sometimes expensive to upgrade, and may create public concerns and negative public reaction. The low cost of gravity wastewater conveyance and the higher costs of building, operating, and maintaining lift stations means that wastewater pumping should be avoided, if possible and technically feasible.

Wastewater pumping can be eliminated or reduced by selecting alternative sewer routes or extending a gravity sewer using direction drilling or other state-of-the-art deep excavation methods. If such alternatives are viable, a cost benefit analysis can determine if a lift station is the most viable choice.

Design Criteria
Cost effective lift stations are designed to: (1) match pump capacity, type, and configuration with wastewater quantity and quality; (2) provide reliable and uninterruptible operation; (3) allow for easy operation and maintenance of the installed equipment; (4) accommodate future capacity expansion; (5) avoid septic conditions and excessive release of odors in the collection system and at the lift station; (6) minimize environmental and landscape impacts on the surrounding residential and commercial developments; and (7) avoid flooding of the lift station and the surrounding areas.

Wet-well
Wet-well design depends on the type of lift station configuration (submersible or dry-well) and the type of pump controls (constant or variable speed). Wet-wells are typically designed large enough to prevent rapid pump cycling but small enough to prevent a long detention time and associated odor release.

Wet-well maximum detention time in constant speed pumps is typically 20 to 30 minutes. Use of variable frequency drives for pump speed control allows wet-well detention time reduction to 5 to 15 minutes. The minimum recommended wet-well bottom slope is to 2:1 to allow self-cleaning and minimum deposit of debris. Effective volume of the wet-well may include sewer pipelines, especially when variable speed drives are used. Wet-wells should always hold some level of sewage to minimize odor release. Bar screens or grinders are often installed in or upstream of the wet-well to minimize pump clogging problems.

Wastewater Pumps

The number of wastewater pumps and associated capacity should be selected to provide head capacity characteristics that correspond as nearly as possible to wastewater quantity fluctuations. His can be accomplished by preparing pump/pipeline system head-capacity curves showing all conditions of head (elevation of a free surface of water) and capacity under which the pumps will be required to operate.

The number of pumps to be installed in a lift station depends on the station capacity, the range of flow and the regulations. In small stations, with maximum inflows of less than 700 gallons per minute), two pumps are customarily installed, with each unit able to meet the maximum influent rate. For larger lift stations, the size and number of pumps should be selected so that the range of influent flow rates can be met without starting and stopping pumps too frequently and without excessive wet-well storage.

Depending on the system, the pumps are designed to run at a reduced rate. The pumps may also alternate to equalize wear and tear. Additional pumps may provide intermediate capacities better matched to typical daily flows. An alternative option is to provide flow flexibility with variable speed pumps.

For pump stations with high head-losses, the single pump flow approach is usually the most suitable. Parallel pumping is not as effective for such stations because two pumps operating together yield only slightly higher flows than one pump. If the peak flow is to be achieved with multiple pumps in parallel, the lift station must be equipped with at least three pumps: two duty pumps that together provide peak flow and one standby pump for emergency backup.

Parallel peak pumping is typically used in large lift stations with relatively flat system head curves. Such curves allow multiple pumps to deliver substantially more flow than a single pump. The use of multiple pumps in parallel provides more flexibility. Several types of centrifugal pumps are used in wastewater lift stations. In the straight-flow centrifugal pumps, wastewater does not change direction as it passes through the pumps and into the discharge pipe. These pumps are well suited for low-flow/high head conditions.

In angle-flow pumps, wastewater enters the impeller axially and passes through the volute casing at 90 degrees to its original direction. This type of pump is appropriate for pumping against low or moderate heads. Mixed flow pumps are most viable for pumping large quantities of wastewater at low head. In these pumps, the outside diameter of the impeller is less than an ordinary centrifugal pump, increasing flow volume.

Ventilation
Ventilation and heating are required if the lift station includes an area routinely entered by personnel. Ventilation is particularly important to prevent the collection of toxic and/or explosive gases. According to the Nation Fire Protection Association (NFPA) Section 820, all continuous ventilation systems should be fitted with flow detection devices connected to alarm systems to indicate ventilation system failure. Dry-well ventilation codes typically require six continuous air changes per hour or 30 intermittent air changes per hour. Wet-wells typically require 12 continuous air changes per hour or 60 intermittent air changes per hour. Motor control center (MCC) rooms should have a ventilation system adequate to provide six air changes per hour and should be air conditioned to between 13 and 32 degrees Celsius (55 to 90 degrees F). If the control room is combined with an MCC room, the temperature should not exceed 30 degrees C or 85 degrees F. All other spaces should be designed for 12 air changes per hour. The minimum temperature should be 13 degrees C (55 degrees F) whenever chemicals are stored or used.

Odor Control
Odor control is frequently required for lift stations. A relatively simple and widely used odor control alternative is minimizing wet-well turbulence. More effective options include collection of odors generated at the lift station and treating them in scrubbers or biofilters or the addition of odor control chemicals to the sewer upstream of the lift station. Chemicals typically used for odor control include chlorine, hydrogen peroxide, metal salts (ferric chloride and ferrous sulfate) oxygen, air, and potassium permanganate. Chemicals should be closely monitored to avoid affecting downstream treatment processes, such as extended aeration.

Power Supply
The reliability of power for the pump motor drives is a basic design consideration. Commonly used methods of emergency power supply include electric power feed from two independent power distribution lines; an on-site standby generator; an adequate portable generator with quick connection; a stand-by engine driven pump; ready access to a suitable portable pumping unit and appropriate connections; and availability of an adequate holding facility for wastewater storage upstream of the lift station.

Performance
The overall performance of a lift station depends on the performance of the pumps. All pumps have four common performance characteristics: capacity, head, power, and overall efficiency. Capacity (flow rate) is the quantity of liquid pumped per unit of time, typically measured as gallons per minute (gpm) or million gallons per day (mgd).

Head is the energy supplied to the wastewater per unit weight, typically expressed as feet of water. Power is the energy consumed by a pump per unit time, typically measured as kilowatt-hours. Overall efficiency is the ratio of useful hydraulic work performed to actual work input. Efficiency reflects the pump relative power losses and is usually measured as a percentage of applied power.

Pump performance curves are used to define and compare the operating characteristics of a pump and to identify the best combination of performance characteristics under which a lift station pumping system will operate under typical conditions (flows and heads). Pump systems operate at 75 to 85 percent efficiency most of the time, while overall pump efficiency depends on the type of installed pumps, their control system, and the fluctuation of influent wastewater flow.

Performance optimization strategies focus on different ways to match pump operational characteristics with system flow and head requirements. They may include the following options: adjusting system flow paths installing variable speed drives; using parallel pumps installing pumps of different sizes trimming a pump impeller; or putting a two-speed motor on one or more pumps in a lift station. Optimizing system performance may yield significant electrical energy savings.

Operation and Maintenance

Lift station operation is usually automated and does not require continuous on-site operator presence. However, frequent inspections are recommended to ensure normal functioning and to identify potential problems. Lift station inspection typically includes observation of pumps, motors and drives for unusual noise, vibration, heating and leakage, check of pump suction and discharge lines for valving arrangement and leakage, check of control panel switches for proper position, monitoring of discharge pump rates and pump speed, and monitoring of the pump suction and discharge pressure.

Weekly inspections are typically conducted, although the frequency really depends on the size of the lift station. If a lift station is equipped with grinder bar screens to remove coarse materials from the wastewater, these materials are collected in containers and disposed of to a sanitary landfill site as needed. If the lift station has a scrubber system for odor control, chemicals are supplied and replenished typically every three months. If chemicals are added for odor control ahead of the lift station, the chemical feed stations should be inspected weekly and chemicals replenished as needed.

The most labor-intensive task for lift stations is routine preventive maintenance. A well-planned maintenance program for lift station pumps prevents unnecessary equipment wear and downtime. Lift station operators must maintain an inventory of critical spare parts. The number of spare parts in the inventory depends on the critical needs of the unit, the rate at which the part normally fails, and the availability of the part. The operator should tabulate each pumping element in the system and its recommended spare parts. This information is typically available from the operation and maintenance manuals provided with the lift station.

Operating Costs
Lift station costs depend on many factors, including
(1) wastewater quality, quantity, and projections;
(2) zoning and land use planning of the area where the lift station will be located;
(3) alternatives for standby power sources;
(4) operation and maintenance needs and support;
(5) soil properties and underground conditions;
(6) required lift to the receiving (discharge) sewer line;
(7) the severity of impact of accidental sewage spill upon the local area; and
(8) the need for an odor control system.

These site and system specific factors must be examined and incorporated in preparing a lift station cost estimate.

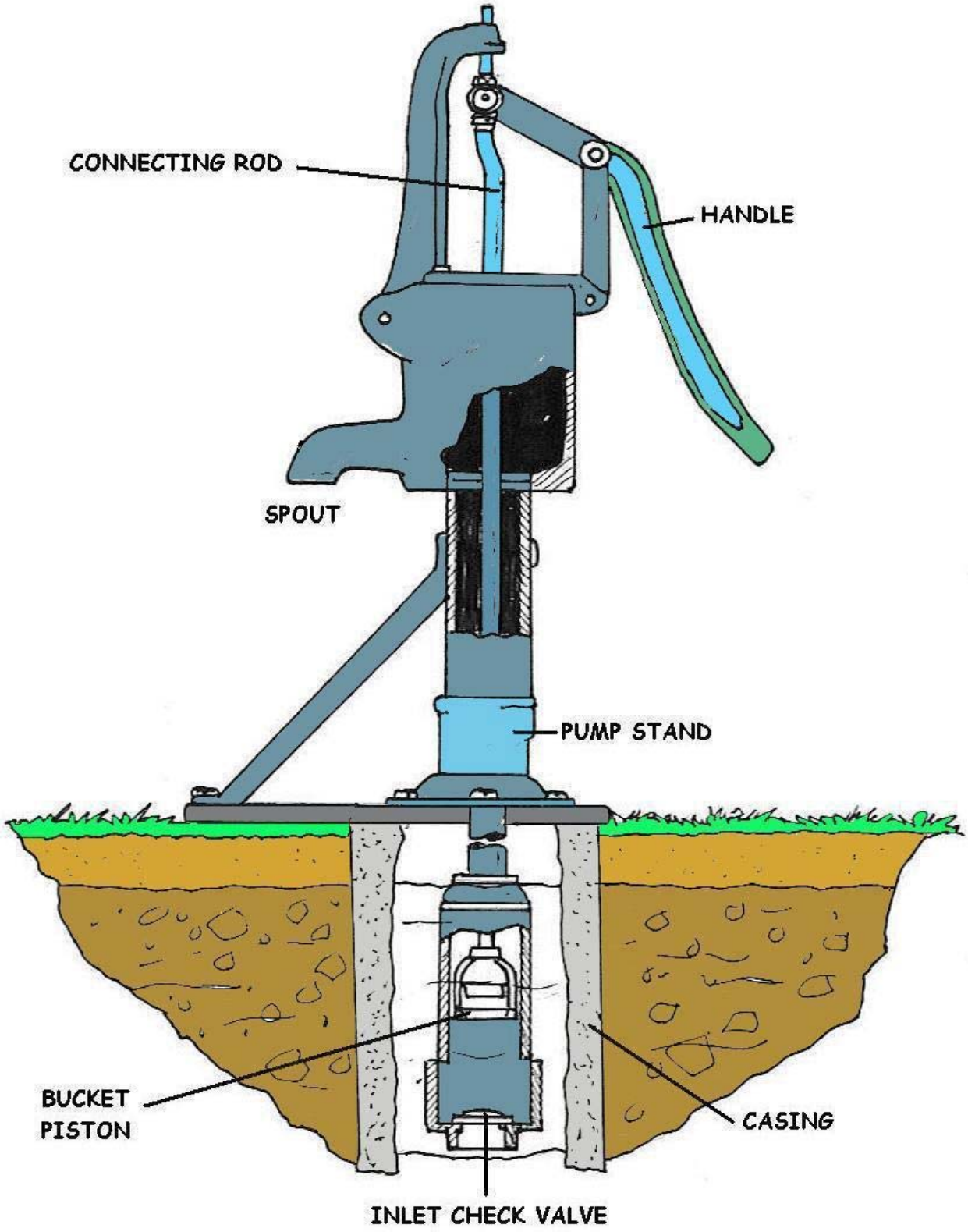
Construction Costs
The most important factors influencing cost are the design lift station capacity and the installed pump power. Another cost factor is the lift station complexity. Factors which classify a lift station as complex include two or more of the following:
(1) extent of excavation;
(2) congested site and/or restricted access;
(3) rock excavation;
(4) extensive dewatering requirements, such as cofferdams;
(5) site conflicts, including modification or removal of existing facilities;
(6) special foundations, including piling;
(7) dual power supply and on-site switch stations and emergency power generator; and
(8) high pumping heads (design heads in excess of 200 ft).

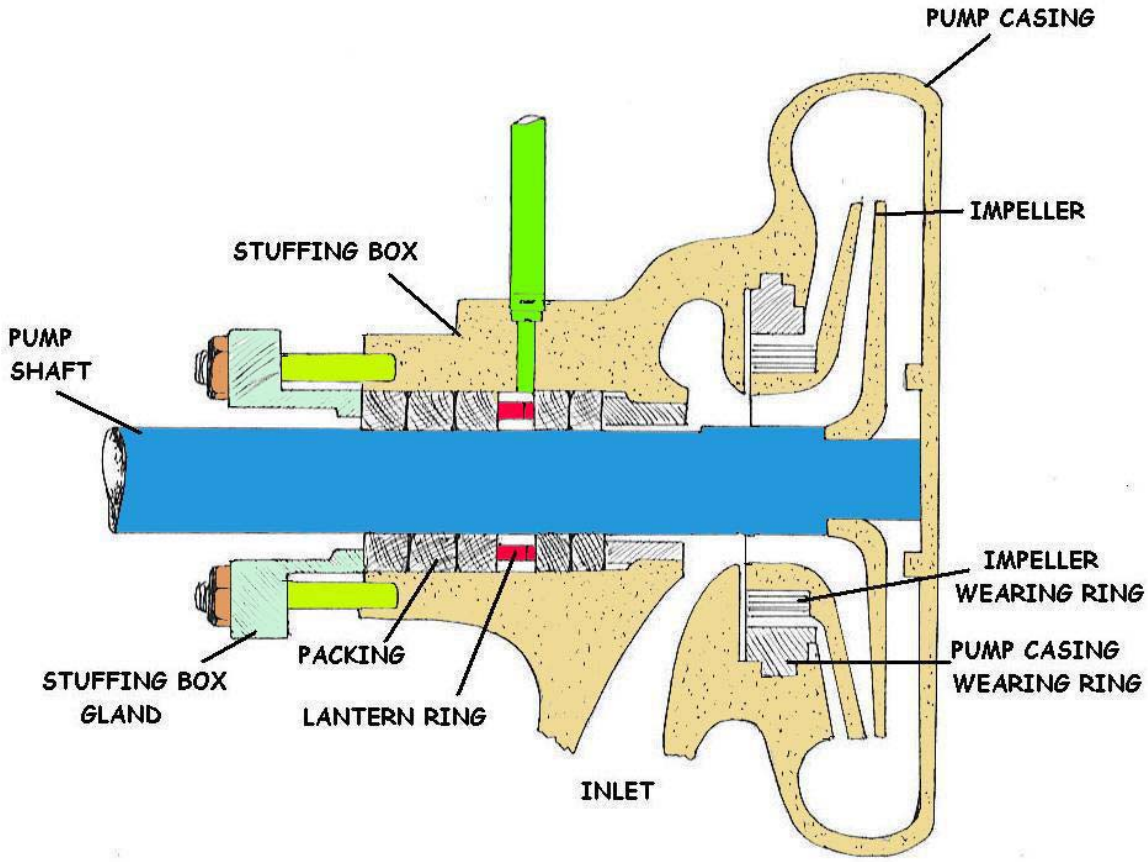
Mechanical, electrical, and control equipment delivered to a pumping station construction site typically account for 15 to 30 percent of total construction costs. Lift station construction has a significant economy-of-scale. Typically, if the capacity of a lift station is increased 100 percent, the construction cost would increase only 50 to 55 percent. An important consideration is that two identical lift stations will cost 25 to 30 percent more than a single station of the same combined capacity. Usually, complex lift stations cost two to three times more than more simple lift stations with no construction complications.

Operation and Maintenance Costs

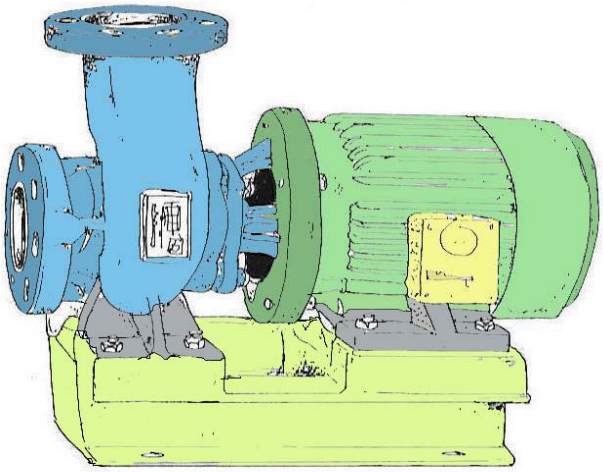
Lift station operation and maintenance costs include power, labor, maintenance, and chemicals (if used for odor control). Usually, the costs for solids disposal are minimal, but are included if the lift station is equipped with bar screens to remove coarse materials from the wastewater. Typically, power costs account for 85 to 95 percent of the total operation and maintenance costs and are directly proportional to the unit cost of power and the actual power used by the lift station pumps. Labor costs average 1 to 2 percent of total costs. Annual maintenance costs vary, depending on the complexity of the equipment and instrumentation.

Pump, Motor and Hydraulic Section





A centrifugal pump has two main components:
I. A rotating component comprised of an impeller and a shaft
II. A stationary component comprised of a casing, casing cover, and bearings.



END SUCTION CENTRIFUGAL PUMP

Common Hydraulic Terms

Head
The height of a column or body of fluid above a given point expressed in linear units. Head is often used to indicate gauge pressure. Pressure is equal to the height times the density of the liquid.

Head, Friction
The head required to overcome the friction at the interior surface of a conductor and between fluid particles in motion. It varies with flow, size, type, and conditions of conductors and fittings, and the fluid characteristics.

Head, static
The height of a column or body of fluid above a given point.

Hydraulics
Engineering science pertaining to liquid pressure and flow.

Hydrokinetics
Engineering science pertaining to the energy of liquid flow and pressure.

Pascal's Law
A pressure applied to a confined fluid at rest is transmitted with equal intensity throughout the fluid.

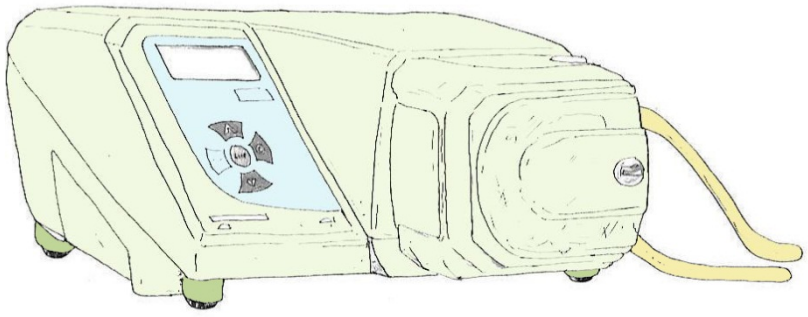
Pressure
The application of continuous force by one body upon another that it is touching; compression. Force per unit area, usually expressed in pounds per square inch (Pascal or bar).

Pressure, Absolute
The pressure above zone absolute, i.e. the sum of atmospheric and gauge pressure. In vacuum related work it is usually expressed in millimeters of mercury. (mmHg).

Pressure, Atmospheric
Pressure exported by the atmosphere at any specific location. (Sea level pressure is approximately 14.7 pounds per square inch absolute, 1 bar = 14.5psi.)

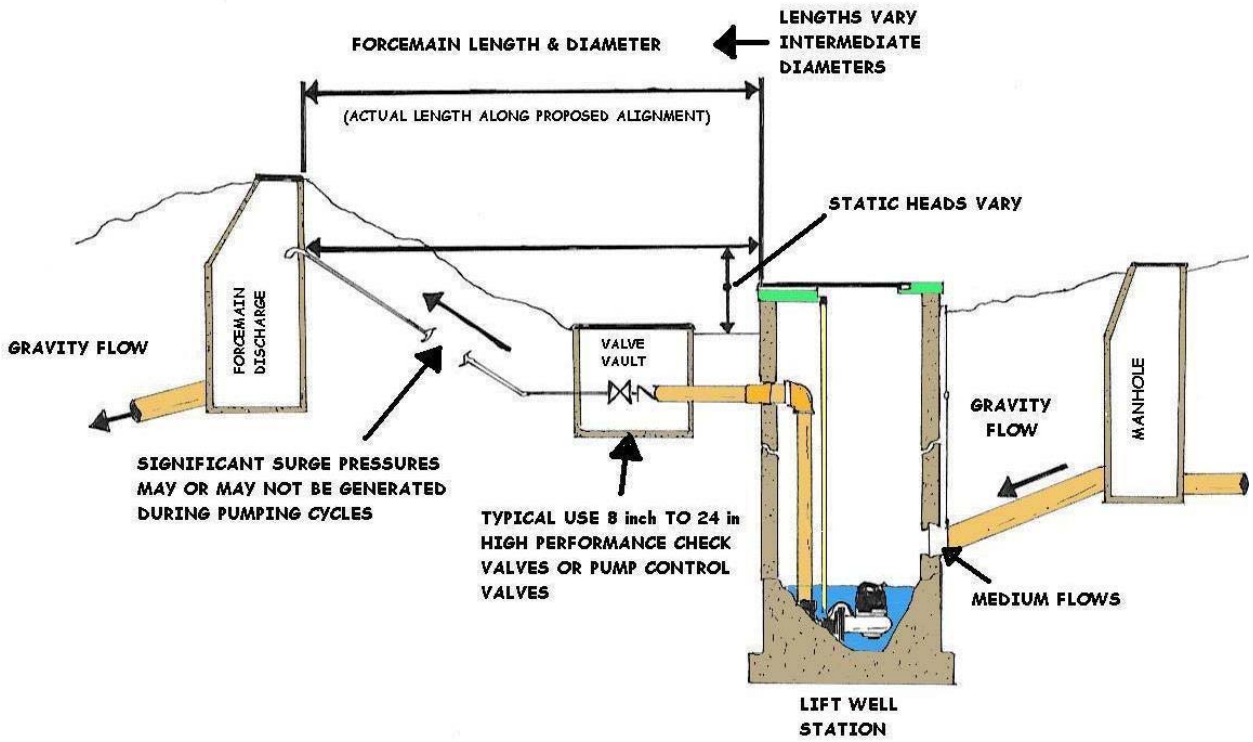
Pressure, Gauge
Pressure differential above or below ambient atmospheric pressure.

Pressure, Static
The pressure in a fluid at rest.



PERISTALTIC PUMP

MEDIUM SEWAGE LIFT STATION
TYPICAL CHARACTERISTICS



Hydraulic Principles Section

Definition: **Hydraulics** is a branch of engineering concerned mainly with moving liquids. The term is applied commonly to the study of the mechanical properties of water, other liquids, and even gases when the effects of compressibility are small. Hydraulics can be divided into two areas, hydrostatics and hydrokinetics.

Hydraulics: ***The Engineering science pertaining to liquid pressure and flow.***
The word ***hydraulics*** is based on the Greek word for water, and originally covered the study of the physical behavior of water at rest and in motion. Use has broadened its meaning to include the behavior of all liquids, although it is primarily concerned with the motion of liquids. Hydraulics includes the manner in which liquids act in tanks and pipes, deals with their properties, and explores ways to take advantage of these properties.

Hydrostatics, the consideration of liquids at rest, involves problems of buoyancy and flotation, pressure on dams and submerged devices, and hydraulic presses. The relative incompressibility of liquids is one of its basic principles. Hydrodynamics, the study of liquids in motion, is concerned with such matters as friction and turbulence generated in pipes by flowing liquids, the flow of water over weirs and through nozzles, and the use of hydraulic pressure in machinery.

Hydrostatics
Hydrostatics is about the pressures exerted by a fluid at rest. Any fluid is meant, not just water. Research and careful study on water yields many useful results of its own, however, such as forces on dams, buoyancy and hydraulic actuation, and is well worth studying for such practical reasons. Hydrostatics is an excellent example of deductive mathematical physics, one that can be understood easily and completely from a very few fundamentals, and in which the predictions agree closely with experiment.

There are few better illustrations of the use of the integral calculus, as well as the principles of ordinary statics, available to the student. A great deal can be done with only elementary mathematics. Properly adapted, the material can be used from the earliest introduction of school science, giving an excellent example of a quantitative science with many possibilities for hands-on experiences.

The definition of a fluid deserves careful consideration. Although time is not a factor in hydrostatics, it enters in the approach to hydrostatic equilibrium. It is usually stated that a fluid is a substance that cannot resist a shearing stress, so that pressures are normal to confining surfaces. Geology has now shown us clearly that there are substances which can resist shearing forces over short time intervals, and appear to be typical solids, but which flow like liquids over long time intervals. Such materials include wax and pitch, ice, and even rock.

A ball of pitch, which can be shattered by a hammer, will spread out and flow in months. Ice, a typical solid, will flow in a period of years, as shown in glaciers, and rock will flow over hundreds of years, as in convection in the mantle of the earth.

Shear earthquake waves, with periods of seconds, propagate deep in the earth, though the rock there can flow like a liquid when considered over centuries. The rate of shearing may not be strictly proportional to the stress, but exists even with low stress.

Viscosity may be the physical property that varies over the largest numerical range, competing with electrical resistivity. There are several familiar topics in hydrostatics which often appears in expositions of introductory science, and which are also of historical interest and can enliven their presentation. Let's start our study with the principles of our atmosphere.

Atmospheric Pressure

The atmosphere is the entire mass of air that surrounds the earth. While it extends upward for about 500 miles, the section of primary interest is the portion that rests on the earth's surface and extends upward for about 7 1/2 miles. This layer is called the troposphere.

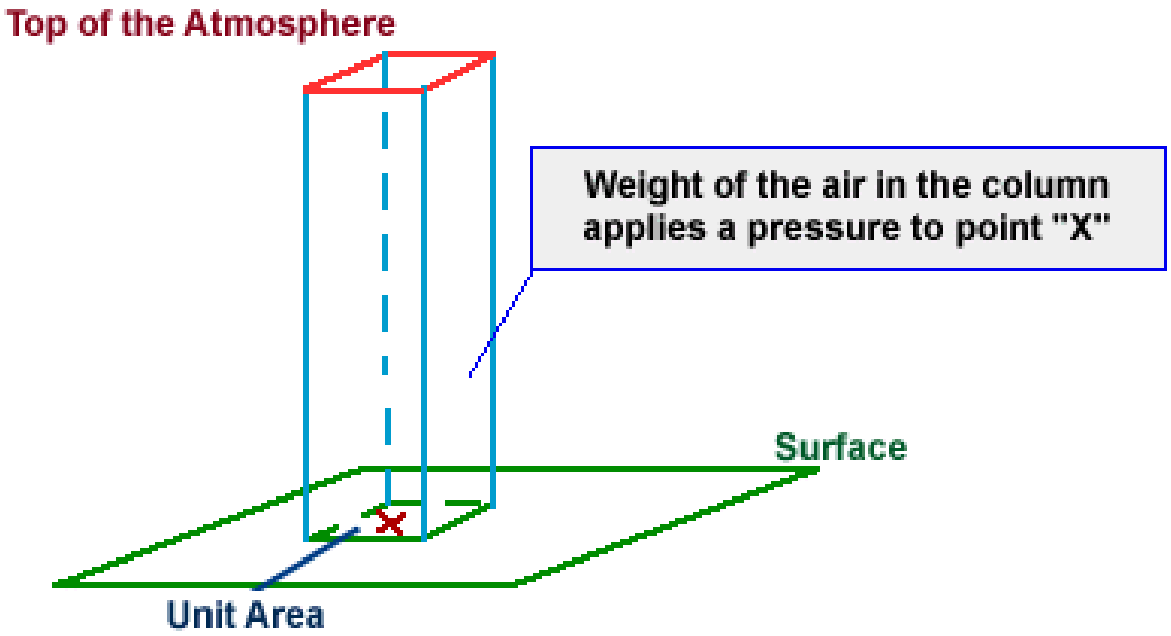
If a column of air 1-inch square extending all the way to the "***top***" of the atmosphere could be weighed, this column of air would weigh approximately 14.7 pounds at sea level. Thus, atmospheric pressure at sea level is approximately 14.7 psi.

As one ascends, the atmospheric pressure decreases by approximately 1.0 psi for every 2,343 feet. However, below sea level, in excavations and depressions, atmospheric pressure increases. Pressures under water differ from those under air only because the weight of the water must be added to the pressure of the air.

Atmospheric pressure can be measured by any of several methods. The common laboratory method uses the mercury column barometer. The height of the mercury column serves as an indicator of atmospheric pressure. At sea level and at a temperature of 0° Celsius (C), the height of the mercury column is approximately 30 inches, or 76 centimeters. This represents a pressure of approximately 14.7 psi. The 30-inch column is used as a reference standard.

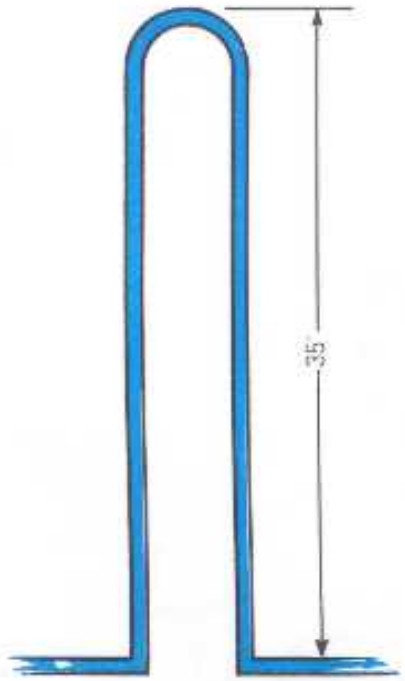
Another device used to measure atmospheric pressure is the aneroid barometer. The aneroid barometer uses the change in shape of an evacuated metal cell to measure variations in atmospheric pressure. The thin metal of the aneroid cell moves in or out with the variation of pressure on its external surface. This movement is transmitted through a system of levers to a pointer, which indicates the pressure.

The atmospheric pressure does not vary uniformly with altitude. It changes very rapidly. Atmospheric pressure is defined as the force per unit area exerted against a surface by the weight of the air above that surface. In the diagram on the following page, the pressure at point "**X**" increases as the weight of the air above it increases. The same can be said about decreasing pressure, where the pressure at point "**X**" decreases if the weight of the air above it also decreases.



Barometric Loop

The barometric loop consists of a continuous section of supply piping that abruptly rises to a height of approximately 35 feet and then returns back down to the originating level. It is a loop in the piping system that effectively protects against backsiphonage. It may not be used to protect against back-pressure.



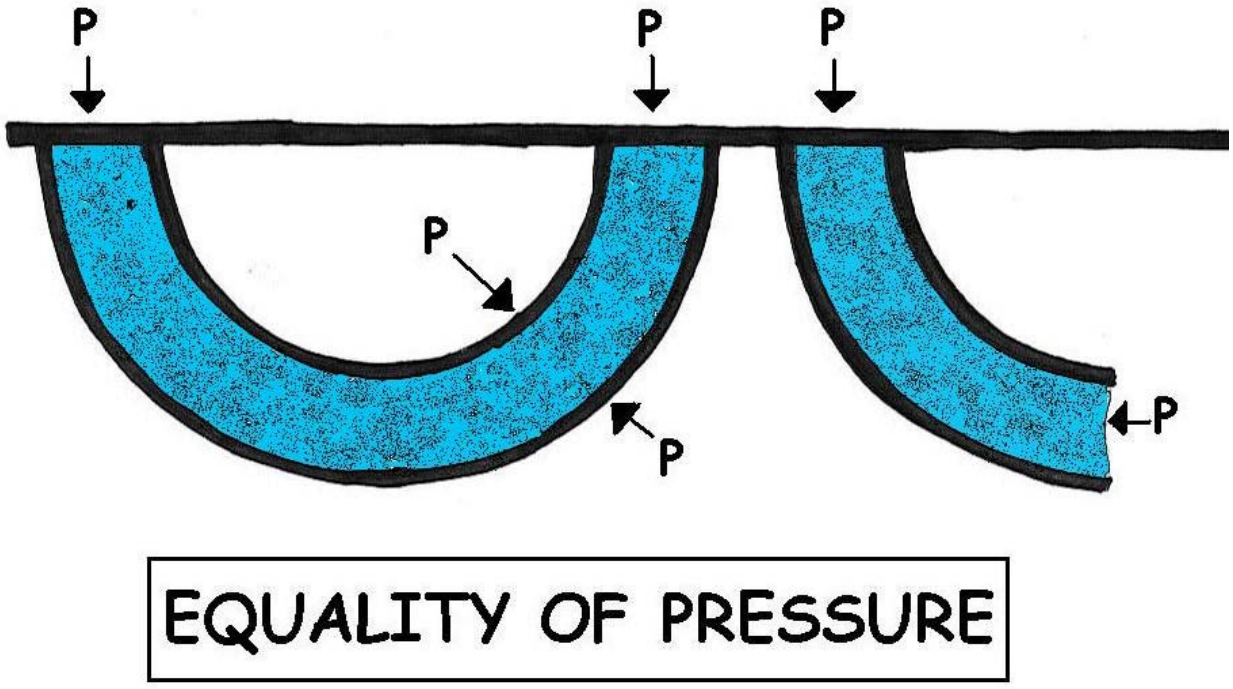
Its operation, in the protection against backsiphonage, is based upon the principle that a water column, at sea level pressure, will not rise above 33.9 feet. In general, barometric loops are locally fabricated, and are 35 feet high.

Pressure may be referred to using an absolute scale, pounds per square inch absolute (**psia**), or gauge scale, (**psiag**). Absolute pressure and gauge pressure are related. Absolute pressure is equal to gauge pressure plus the atmospheric pressure. At sea level, the atmospheric pressure is 14.7 psai.

Absolute pressure is the total pressure. Gauge pressure is simply the pressure read on the gauge. If there is no pressure on the gauge other than atmospheric, the gauge will read zero. Then the absolute pressure would be equal to 14.7 psi, which is the atmospheric pressure.

Pressure

By a fluid, we have a material in mind like water or air, two very common and important fluids. Water is incompressible, while air is very compressible, but both are fluids. Water has a definite volume; air does not. Water and air have low viscosity; that is, layers of them slide very easily on one another, and they quickly assume their permanent shapes when disturbed by rapid flows. Other fluids, such as molasses, may have high viscosity and take a long time to come to equilibrium, but they are no less fluids. The coefficient of viscosity is the ratio of the shearing force to the velocity gradient. Hydrostatics deals with permanent, time-independent states of fluids, so viscosity does not appear, except as discussed in the Introduction.



EQUALITY OF PRESSURE

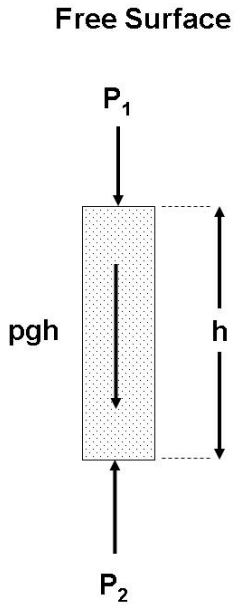
A fluid, therefore, is a substance that cannot exert any permanent forces tangential to a boundary. Any force that it exerts on a boundary must be normal to the boundary. Such a force is proportional to the area on which it is exerted, and is called a pressure. We can imagine any surface in a fluid as dividing the fluid into parts pressing on each other, as if it were a thin material membrane, and so think of the pressure at any point in the fluid, not just at the boundaries. In order for any small element of the fluid to be in equilibrium, the pressure must be the same in all directions (or the element would move in the direction of least pressure), and if no other forces are acting on the body of the fluid, the pressure must be the same at all neighboring points.

Therefore, in this case the pressure will be the same throughout the fluid, and the same in any direction at a point (Pascal's Principle). Pressure is expressed in units of force per unit area such as dyne/cm^2, N/cm^2 (pascal), pounds/in^2 (psi) or pounds/ft^2 (psf). The axiom that if a certain volume of fluid were somehow made solid, the equilibrium of forces would not be disturbed, is useful in reasoning about forces in fluids.

On earth, fluids are also subject to the force of gravity, which acts vertically downward, and has a magnitude $\gamma = \rho g$ per unit volume, where g is the acceleration of gravity, approximately 981 cm/s^2 or 32.15 ft/s^2, ρ is the density, the mass per unit volume, expressed in g/cm^3, kg/m^3, or $slug/ft^3$, and γ is the specific weight, measured in lb/in^3, or lb/ft^3 (pcf). Gravitation is an example of a body force that disturbs the equality of pressure in a fluid. The presence of the gravitational body force causes the pressure to increase with depth, according to the equation $dp = \rho g\, dh$, in order to support the water above. We call this relation the barometric equation, for when this equation is integrated, we find the variation of pressure with height or depth. If the fluid is incompressible, the equation can be integrated at once, and the pressure as a function of depth h is $p = \rho gh + p0$.

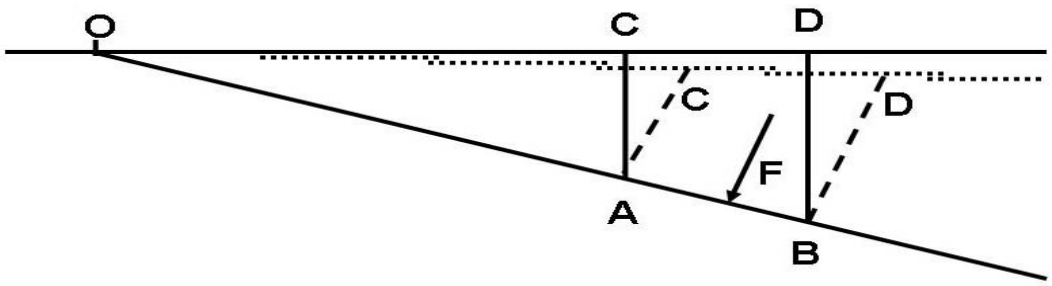
The density of water is about 1 g/cm^3, or its specific weight is 62.4 pcf. We may ask what depth of water gives the normal sea-level atmospheric pressure of 14.7 psi, or 2117 psf.

This is simply 2117 / 62.4 = 33.9 ft of water. This is the maximum height to which water can be raised by a suction pump, or, more correctly, can be supported by atmospheric pressure. Professor James Thomson (brother of William Thomson, Lord Kelvin) illustrated the equality of pressure by a "curtain-ring" analogy shown in the diagram. A section of the toroid was identified, imagined to be solidified, and its equilibrium was analyzed.



Increase of Pressure with Depth

The forces exerted on the curved surfaces have no component along the normal to a plane section, so the pressures at any two points of a plane must be equal, since the fluid represented by the curtain ring was in equilibrium. The right-hand part of the diagram illustrates the equality of pressures in orthogonal directions. This can be extended to any direction whatever, so Pascal's Principle is established. This demonstration is similar to the usual one using a triangular prism and considering the forces on the end and lateral faces separately.



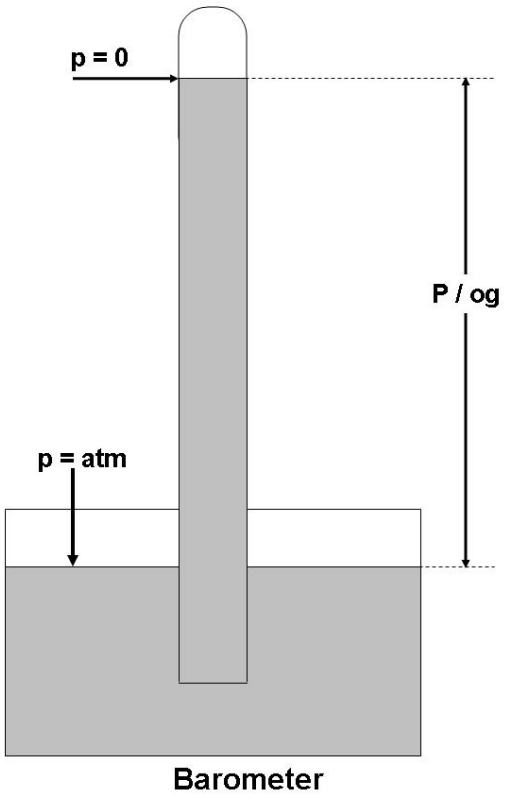
Thrust on a Plane

Free Surface Perpendicular to Gravity

When gravity acts, the liquid assumes a free surface perpendicular to gravity, which can be proved by Thomson's method. A straight cylinder of unit cross-sectional area (assumed only for ease in the arithmetic) can be used to find the increase of pressure with depth. Indeed, we see that p2 = p1 + ρgh. The upper surface of the cylinder can be placed at the free surface if desired. The pressure is now the same in any direction at a point, but is greater at points that lie deeper. From this same figure, it is easy to prove Archimedes' Principle that the buoyant force is equal to the weight of the displaced fluid, and passes through the center of mass of this displaced fluid.

Geometric Arguments

Ingenious geometric arguments can be used to substitute for easier, but less transparent arguments using calculus. For example, the force acting on one side of an inclined plane surface whose projection is AB can be found as in the diagram on the previous page. O is the point at which the prolonged projection intersects the free surface. The line AC' perpendicular to the plane is made equal to the depth AC of point A, and line BD' is similarly drawn equal to BD. The line OD' also passes through C', by proportionality of triangles OAC' and OAD'. Therefore, the thrust F on the plane is the weight of a prism of fluid of cross-section AC'D'B, passing through its centroid normal to plane AB. Note that the thrust is equal to the density times the area times the depth of the center of the area; its line of action does not pass through the center, but below it, at the center of thrust. The same result can be obtained with calculus by summing the pressures and the moments.



Atmospheric Pressure and its Effects

Suppose a vertical pipe is stood in a pool of water, and a vacuum pump applied to the upper end. Before we start the pump, the water levels outside and inside the pipe are equal, and the pressures on the surfaces are also equal and are equal to the atmospheric pressure.

Now start the pump. When it has sucked all the air out above the water, the pressure on the surface of the water inside the pipe is zero, and the pressure at the level of the water on the outside of the pipe is still the atmospheric pressure.

Of course, there is the vapor pressure of the water to worry about if you want to be precise, but we neglect this complication in making our point. We require a column of water 33.9 ft high inside the pipe, with a vacuum above it, to balance the atmospheric pressure. Now do the same thing with liquid mercury, whose density at 0 °C is 13.5951 times that of water. The height of the column is 2.494 ft, 29.92 in, or 760.0 mm.

Standard Atmospheric Pressure

This definition of the standard atmospheric pressure was established by Regnault in the mid-19th century. In Britain, 30 in. Hg (inches of mercury) had been used previously. As a practical matter, it is convenient to measure pressure differences by measuring the height of liquid columns, a practice known as manometry. The barometer is a familiar example of this, and atmospheric pressures are traditionally given in terms of the length of a mercury column. To make a barometer, the barometric tube, closed at one end, is filled with mercury and then inverted and placed in a mercury reservoir. Corrections must be made for temperature, because the density of mercury depends on the temperature, and the brass scale expands for capillarity if the tube is less than about 1 cm in diameter, and even slightly for altitude, since the value of g changes with altitude.

The vapor pressure of mercury is only 0.001201 mmHg at 20°C, so a correction from this source is negligible. For the usual case of a mercury column (α = 0.000181792 per °C) and a brass scale (&alpha = 0.0000184 per °C) the temperature correction is -2.74 mm at 760 mm and 20°C. Before reading the barometer scale, the mercury reservoir is raised or lowered until the surface of the mercury just touches a reference point, which is mirrored in the surface so it is easy to determine the proper position.

An aneroid barometer uses a partially evacuated chamber of thin metal that expands and contracts according to the external pressure. This movement is communicated to a needle that revolves in a dial. The materials and construction are arranged to give a low temperature coefficient. The instrument must be calibrated before use, and is usually arranged to read directly in elevations. An aneroid barometer is much easier to use in field observations, such as in reconnaissance surveys. In a particular case, it would be read at the start of the day at the base camp, at various points in the vicinity, and then finally at the starting point, to determine the change in pressure with time. The height differences can be calculated from h = 60,360 log(P/p) [1 + (T + t - 64)/986) feet, where P and p are in the same units, and T, t are in °F.

An absolute pressure is referring to a vacuum, while a gauge pressure is referring to the atmospheric pressure at the moment. A negative gauge pressure is a (partial) vacuum. When a vacuum is stated to be so many inches, this means the pressure below the atmospheric pressure of about 30 in. A vacuum of 25 inches is the same thing as an absolute pressure of 5 inches (of mercury).

Vacuum

The term ***vacuum*** indicates that the absolute pressure is less than the atmospheric pressure and that the gauge pressure is negative. A complete or total vacuum would mean a pressure of 0 psia or –14.7 psig.

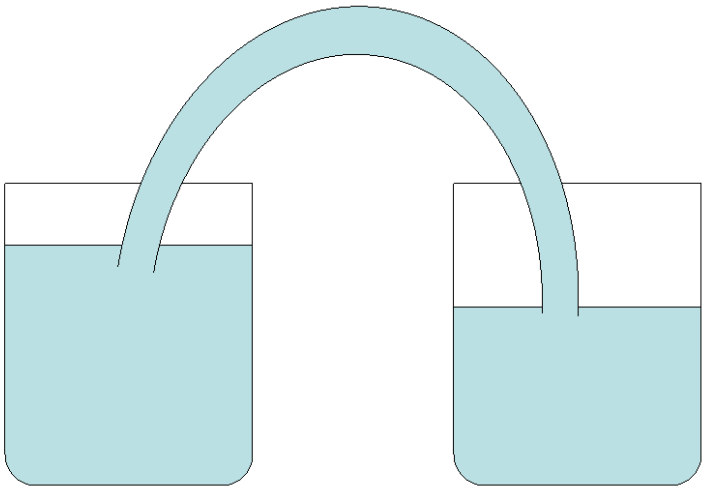
Since it is impossible to produce a total vacuum, the term vacuum, as used in this document, will mean all degrees of partial vacuum. In a partial vacuum, the pressure would range from slightly less than 14.7 psia (0 psig) to slightly greater than 0 psia (-14.7 psig).

Backsiphonage results from atmospheric pressure exerted on a liquid, forcing it toward a supply system that is under a vacuum.

Water Pressure

The weight of a cubic foot of water is 62.4 pounds per square foot. The base can be subdivided into 144-square inches with each subdivision being subjected to a pressure of 0.433 psig. Suppose you placed another cubic foot of water on top of the first cubic foot. The pressure on the top surface of the first cube which was originally atmospheric, or 0 psig, would now be 0.4333 psig as a result of the additional cubic foot of water. The pressure of the base of the first cubic foot would be increased by the same amount of 0.866 psig or two times the original pressure.

Pressures are very frequently stated in terms of the height of a fluid. If it is the same fluid whose pressure is being given, it is usually called "head," and the factor connecting the head and the pressure is the weight density ρg. In the English engineer's system, weight density is in pounds per cubic inch or cubic foot. A head of 10 ft is equivalent to a pressure of 624 psf, or 4.33 psi. It can also be considered an energy availability of ft-lb per lb. Water with a pressure head of 10 ft can furnish the same energy as an equal amount of water raised by 10 ft. Water flowing in a pipe is subject to head loss because of friction.

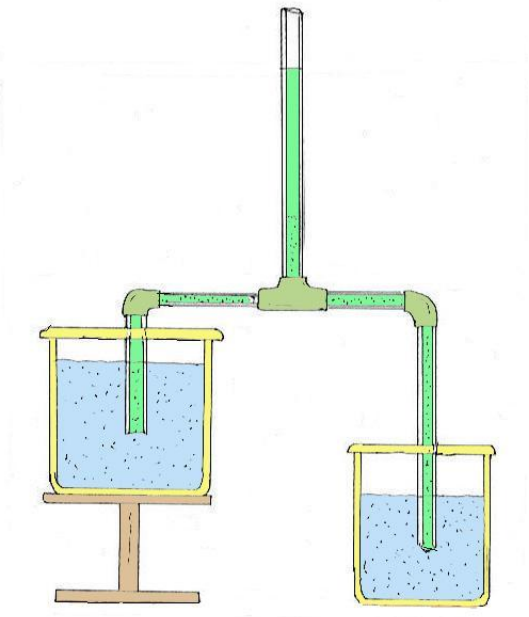


Siphon

Take a jar and a basin of water. Fill the jar with water and invert it under the water in the basin. Now raise the jar as far as you can without allowing its mouth to come above the water surface. It is always a little surprising to see that the jar does not empty itself, but the water remains with no visible means of support. By blowing through a straw, one can put air into the jar, and as much water leaves as air enters.

In fact, this is a famous method of collecting insoluble gases in the chemical laboratory, or for supplying hummingbird feeders. It is good to remind oneself of exactly the balance of forces involved.

Another application of pressure is the siphon. The name is Greek for the tube that was used for drawing wine from a cask. This is a tube filled with fluid connecting two containers of fluid, normally rising higher than the water levels in the two containers, at least to pass over their rims. In the diagram, the two water levels are the same, so there will be no flow. When a siphon goes below the free water levels, it is called an inverted siphon. If the levels in the two basins are not equal, fluid flows from the basin with the higher level into the one with the lower level, until the levels are equal.



PASCAL'S SIPHON

A siphon can be made by filling the tube, closing the ends, and then putting the ends under the surface on both sides. Alternatively, the tube can be placed in one fluid and filled by sucking on it. When it is full, the other end is put in place. The analysis of the siphon is easy, and should be obvious. The pressure rises or falls as described by the barometric equation through the siphon tube. There is obviously a maximum height for the siphon which is the same as the limit of the suction pump, about 34 feet. Inverted siphons are sometimes used in pipelines to cross valleys. Differences in elevation are usually too great to use regular siphons to cross hills, so the fluids must be pressurized by pumps so the pressure does not fall to zero at the crests.

Liquids at Rest

In studying fluids at rest, we are concerned with the transmission of force and the factors which affect the forces in liquids. Additionally, pressure in and on liquids and factors affecting pressure are of great importance.

Pressure and Force

Pressure is the force that pushes water through pipes. Water pressure determines the flow of water from the tap. If pressure is not sufficient then the flow can reduce to a trickle and it will take a long time to fill a kettle or a cistern. The terms ***force*** and ***pressure*** are used extensively in the study of fluid power. It is essential that we distinguish between the terms.

Force means a total push or pull. It is the push or pull exerted against the total area of a particular surface and is expressed in pounds or grams. Pressure means the amount of push or pull (force) applied to each unit area of the surface and is expressed in pounds per square inch (lb/in^2) or grams per square centimeter (gm/cm^2). Pressure maybe exerted in one direction, in several directions, or in all directions.

Computing Force, Pressure, and Area

A formula is used in computing force, pressure, and area in fluid power systems. In this formula, P refers to pressure, F indicates force, and A represents area. Force equals pressure times area. Thus, the formula is written:

General Pumping Fundamentals

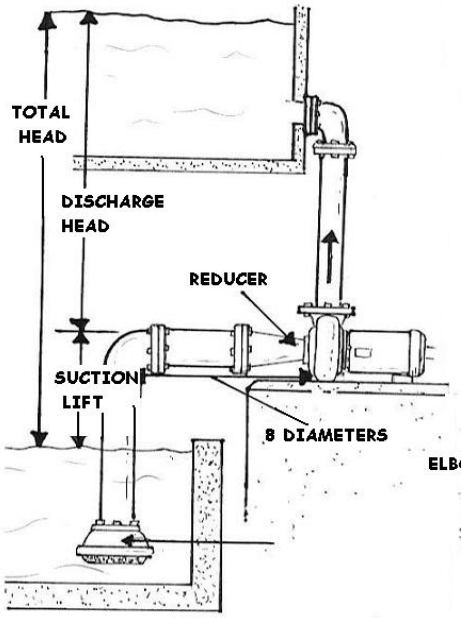


ILLUSTRATION 1

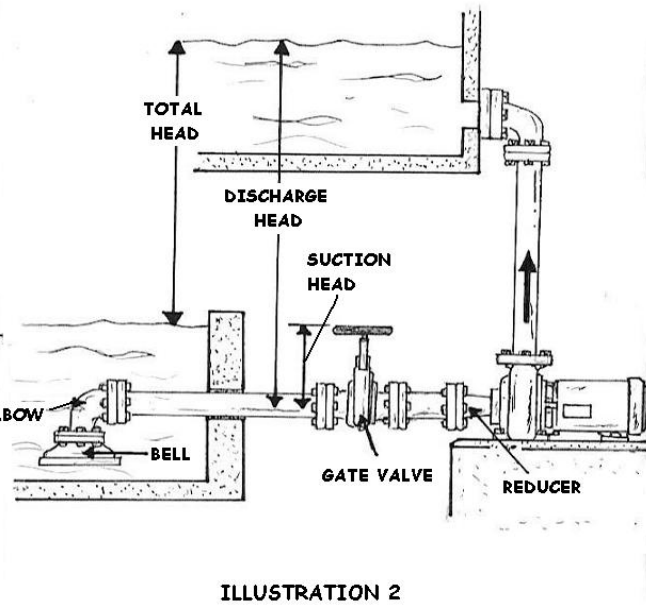


ILLUSTRATION 2

Here are the important points to consider about suction piping when the liquid being pumped is below the level of the pump:

- First, suction lift is when the level of water to be pumped is below the centerline of the pump. Sometimes suction lift is also referred to as '**negative suction head'**.
- The ability of the pump to lift water is the result of a partial vacuum created at the center of the pump.
- This works similar to sucking soda from a straw. As you gently suck on a straw, you are creating a vacuum or a pressure differential. Less pressure is exerted on the liquid inside the straw, so that the greater pressure is exerted on the liquid around the outside of the straw, causing the liquid in the straw to move up. By sucking on the straw, this allows atmospheric pressure to move the liquid.
- Look at the diagram illustrated as "**1**". The foot valve is located at the end of the suction pipe of a pump. It opens to allow water to enter the suction side, but closes to prevent water from passing back out of the bottom end.
- The suction side of pipe should be one diameter larger than the pump inlet. The required eccentric reducer should be turned so that the top is flat and the bottom tapered.

Notice in illustration "**2**" that the liquid is above the level of the pump. Sometimes this is referred to as ***'flooded suction'*** or ***'suction head'*** situations.

Points to Note are:

★ If an elbow and bell are used, they should be at least one pipe diameter from the tank bottom and side. This type of suction piping must have a gate valve which can be used to prevent the reverse flow when the pump has to be removed. In the illustrations you can see in both cases the discharge head is from the centerline of the pump to the level of the discharge water. The total head is the difference between the two liquid levels.

Pump Definitions *(Larger Glossary in the rear of this manual)*

Fluid: Any substance that can be pumped such as oil, water, refrigerant, or even air.

Gasket: Flat material that is compressed between two flanges to form a seal.

Gland follower: A bushing used to compress the packing in the stuffing box and to control leakoff.

Gland sealing line: A line that directs sealing fluid to the stuffing box.

Horizontal pumps: Pumps in which the center line of the shaft is horizontal.

Impeller: The part of the pump that increases the speed of the fluid being handled.

Inboard: The end of the pump closest to the motor.

Inter-stage diaphragm: A barrier that separates stages of a multi-stage pump.

Key: A rectangular piece of metal that prevents the impeller from rotating on the shaft.

Keyway: The area on the shaft that accepts the key.

Kinetic energy: Energy associated with motion.

Lantern ring: A metal ring located between rings of packing that distributes gland sealing fluid.

Leak-off: Fluid that leaks from the stuffing box.

Mechanical seal: A mechanical device that seals the pump stuffing box.

Mixed flow pump: A pump that uses both axial-flow and radial-flow components in one impeller.

Multi-stage pumps: Pumps with more than one impeller.

Outboard: The end of the pump farthest from the motor.

Packing: Soft, pliable material that seals the stuffing box.

Positive displacement pumps: Pumps that move fluids by physically displacing the fluid inside the pump.

Radial bearings: Bearings that prevent shaft movement in any direction outward from the center line of the pump.

Radial flow: Flow at 90° to the center line of the shaft.

Retaining nut: A nut that keeps the parts in place.

Rotor: The rotating parts, usually including the impeller, shaft, bearing housings, and all other parts included between the bearing housing and the impeller.

Score: To cause lines, grooves, or scratches.

Shaft: A cylindrical bar that transmits power from the driver to the pump impeller.

Shaft sleeve: A replaceable tubular covering on the shaft.

Shroud: The metal covering over the vanes of an impeller.

Slop drain: The drain from the area that collects leak-off from the stuffing box.

Slurry: A thick, viscous fluid, usually containing small particles.

Stages: Impellers in a multi-stage pump.

Stethoscope: A metal device that can amplify and pinpoint pump sounds.

Strainer: A device that retains solid pieces while letting liquids through.

Stuffing box: The area of the pump where the shaft penetrates the casing.

Suction: The place where fluid enters the pump.

Suction eye: The place where fluid enters the pump impeller.

Throat bushing: A bushing at the bottom of the stuffing box that prevents packing from being pushed out of the stuffing box into the suction eye of the impeller.

Thrust: Force, usually along the center line of the pump.

Thrust bearings: Bearings that prevent shaft movement back and forth in the same direction as the center line of the shaft.

Troubleshooting: Locating a problem.

Vanes: The parts of the impeller that push and increase the speed of the fluid in the pump.

Vertical pumps: Pumps in which the center line of the shaft runs vertically.

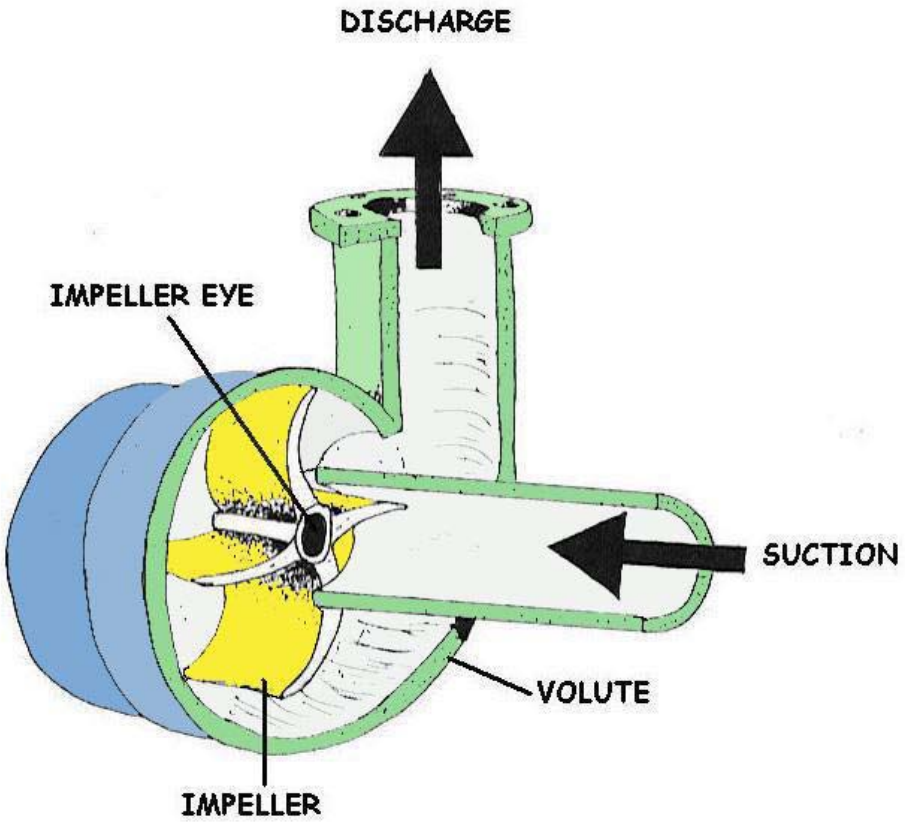
Volute: The part of the pump that changes the speed of the fluid into pressure.

Wearing rings: Replaceable rings on the impeller or the casing that wear as the pump operates.

Single suction volute pump.

Pumps

Pumps are used to move or raise fluids. They are not only very useful, but are excellent examples of hydrostatics. Pumps are of two general types, hydrostatic or positive displacement pumps, and pumps depending on dynamic forces, such as centrifugal pumps. Here we will only consider positive displacement pumps, which can be understood purely by hydrostatic considerations. They have a piston (or equivalent) moving in a closely-fitting cylinder, and forces are exerted on the fluid by motion of the piston.



We have already seen an important example of this in the hydraulic lever or hydraulic press, which we have called quasi-static. The simplest pump is the syringe, filled by withdrawing the piston and emptied by pressing it back in, as its port is immersed in the fluid or removed from it.

More complicated pumps have valves allowing them to work repetitively. These are usually check valves that open to allow passage in one direction, and close automatically to prevent reverse flow. There are many kinds of valves, and they are usually the most trouble-prone and complicated part of a pump. The force pump has two check valves in the cylinder, one for supply and the other for delivery. The supply valve opens when the cylinder volume increases, the delivery valve when the cylinder volume decreases.

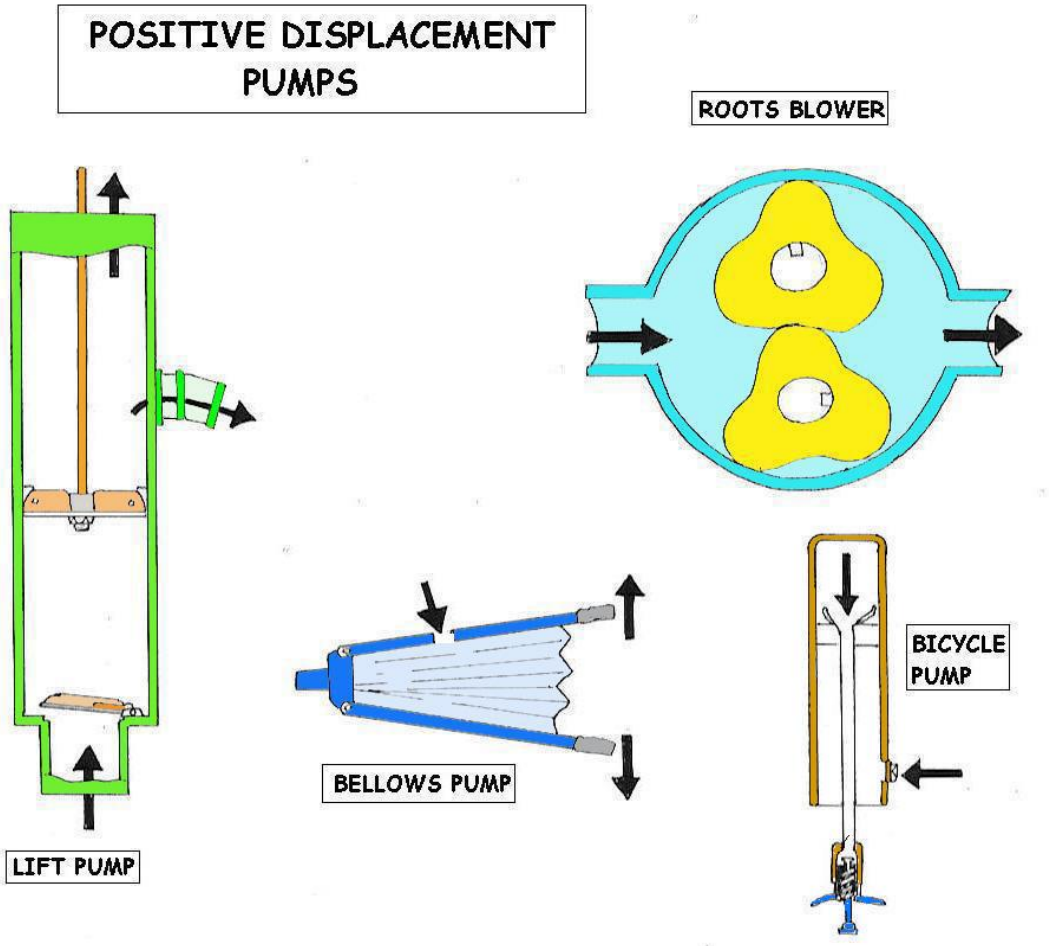
The lift pump has a supply valve and a valve in the piston that allows the liquid to pass around it when the volume of the cylinder is reduced. The delivery in this case is from the upper part of the cylinder, which the piston does not enter.

Diaphragm pumps are force pumps in which the oscillating diaphragm takes the place of the piston. The diaphragm may be moved mechanically, or by the pressure of the fluid on one side of the diaphragm.

Some positive displacement pumps are shown below. The force and lift pumps are typically used for water. The force pump has two valves in the cylinder, while the lift pump has one valve in the cylinder and one in the piston. The maximum lift, or "suction," is determined by the atmospheric pressure, and either cylinder must be within this height of the free surface. The force pump, however, can give an arbitrarily large pressure to the discharged fluid, as in the case of a diesel engine injector. A nozzle can be used to convert the pressure to velocity, to produce a jet, as for fire-fighting. Fire fighting force pumps usually have two cylinders feeding one receiver alternately. The air space in the receiver helps to make the water pressure uniform.

The three pumps below are typically used for air, but would be equally applicable to liquids. The Roots blower has no valves, their place taken by the sliding contact between the rotors and the housing. The Roots blower can either exhaust a receiver or provide air under moderate pressure, in large volumes. The Bellows is a very old device, requiring no accurate machining. The single valve is in one or both sides of the expandable chamber. Another valve can be placed at the nozzle if required. The valve can be a piece of soft leather held close to holes in the chamber. The Bicycle pump uses the valve on the valve stem of the tire or inner tube to hold pressure in the tire. The piston, which is attached to the discharge tube, has a flexible seal that seals when the cylinder is moved to compress the air, but allows air to pass when the movement is reversed.

Diaphragm and vane pumps are not shown, but they act the same way by varying the volume of a chamber, and directing the flow with check valves.



Types of Pumps

The family of pumps comprises a large number of types based on application and capabilities. The two major groups of pumps are dynamic and positive displacement.

Dynamic Pumps (Centrifugal Pump)

Centrifugal pumps are classified into three general categories:
Radial flow—a centrifugal pump in which the pressure is developed wholly by centrifugal force.
Mixed flow—a centrifugal pump in which the pressure is developed partly by centrifugal force and partly by the lift of the vanes of the impeller on the liquid.
Axial flow—a centrifugal pump in which the pressure is developed by the propelling or lifting action of the vanes of the impeller on the liquid.

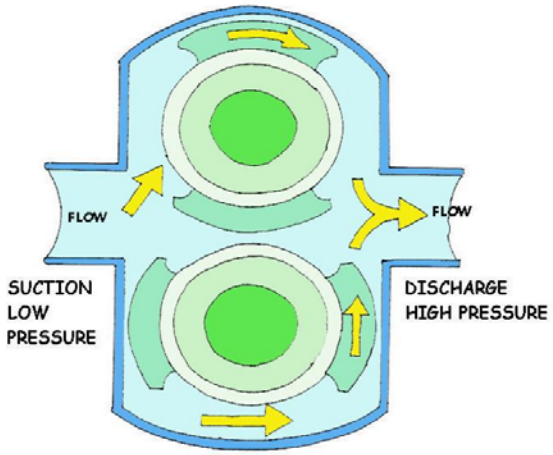
Positive Displacement Pumps

A Positive Displacement Pump has an expanding cavity on the suction side of the pump and a decreasing cavity on the discharge side. Liquid is allowed to flow into the pump as the cavity on the suction side expands and the liquid is forced out of the discharge as the cavity collapses. This principle applies to all types of Positive Displacement Pumps whether the pump is a rotary lobe, gear within a gear, piston, diaphragm, screw, progressing cavity, etc.

A Positive Displacement Pump, unlike a Centrifugal Pump, will produce the same flow at a given RPM no matter what the discharge pressure is. A Positive Displacement Pump cannot be operated against a closed valve on the discharge side of the pump, i.e. it does not have a shut-off head like a Centrifugal Pump does. If a Positive Displacement Pump is allowed to operate against a closed discharge valve it will continue to produce flow which will increase the pressure in the discharge line until either the line bursts or the pump is severely damaged or both.

Types of Positive Displacement Pumps

Single Rotor	Multiple Rotor
Vane	Gear
Piston	Lobe
Flexible Member	Circumferential Piston
Single Screw	Multiple Screw



There are many types of positive displacement pumps. We will look at:

- Plunger pumps
- Diaphragm pumps
- Progressing cavity pumps, and
- Screw pumps

Single Rotator

Component	Description
Vane	The vane(s) may be blades, buckets, rollers, or slippers that cooperate with a dam to draw fluid into and out of the pump chamber.
Piston	Fluid is drawn in and out of the pump chamber by a piston(s) reciprocating within a cylinder(s) and operating port valves.
Flexible Member	Pumping and sealing depends on the elasticity of a flexible member(s) that may be a tube, vane, or a liner.
Single Screw	Fluid is carried between rotor screw threads as they mesh with internal threads on the stator.

Multiple Rotator

Component	Description
Gear	Fluid is carried between gear teeth and is expelled by the meshing of the gears that cooperate to provide continuous sealing between the pump inlet and outlet.
Lobe	Fluid is carried between rotor lobes that cooperate to provide continuous sealing between the pump inlet and outlet.
Circumferential piston	Fluid is carried in spaces between piston surfaces not requiring contacts between rotor surfaces.
Multiple Screw	Fluid is carried between rotor screw threads as they mesh.

What kind of mechanical device do you think is used to provide this positive displacement in the:

Plunger pump?

Diaphragm pump?

In the same way, the progressing cavity and the screw are two other types of mechanical action that can be used to provide movement of the liquid through the pump.

Plunger Pump
The plunger pump is a positive displacement pump that uses a plunger or piston to force liquid from the suction side to the discharge side of the pump. It is used for heavy sludge. The movement of the plunger or piston inside the pump creates pressure inside the pump, so you have to be careful that this kind of pump is never operated against any closed discharge valve. All discharge valves must be open before the pump is started, to prevent any fast build-up of pressure that could damage the pump.

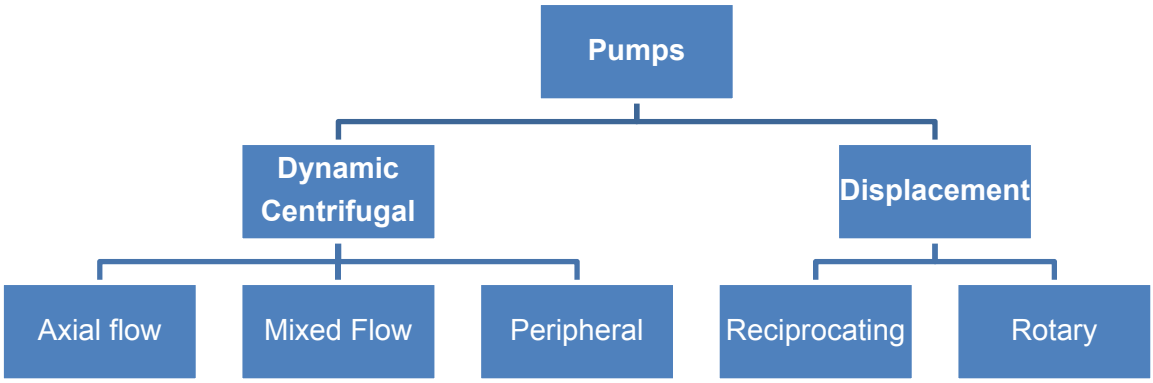
Diaphragm Pumps
In this type of pump, a diaphragm provides the mechanical action used to force liquid from the suction to the discharge side of the pump. The advantage the diaphragm has over the plunger is that the diaphragm pump does not come in contact with moving metal. This can be important when pumping abrasive or corrosive materials.

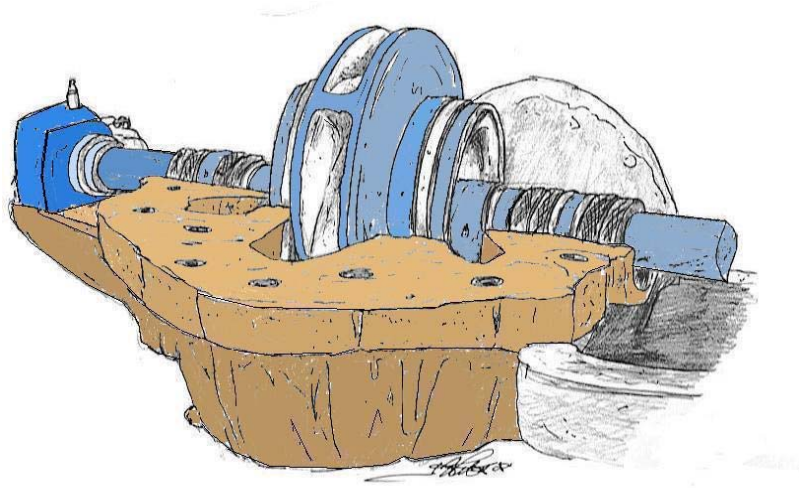
There are three main types of diaphragm pumps available:
1. Diaphragm sludge pump
2. Chemical metering or proportional pump
3. Air-powered double-diaphragm pump

Pump Categories

Let's cover the essentials first. The key to the whole operation is, of course, the *pump.* And regardless of what type it is (reciprocating piston, centrifugal, turbine or jet-ejector, for either shallow or deep well applications), its purpose is to move water and generate the delivery force we call pressure. Sometimes — with centrifugal pumps in particular — pressure is not referred to in pounds per square inch but rather as the equivalent in elevation, called head. No matter; head in feet divided by 2.31 equals pressure, so it's simple enough to establish a common figure.

Pumps may be classified on the basis of the application they serve. All pumps may be divided into two major categories: (1) dynamic, in which energy is continuously added to increase the fluid velocities within the machine, and (2) displacement, in which the energy is periodically added by application of force.



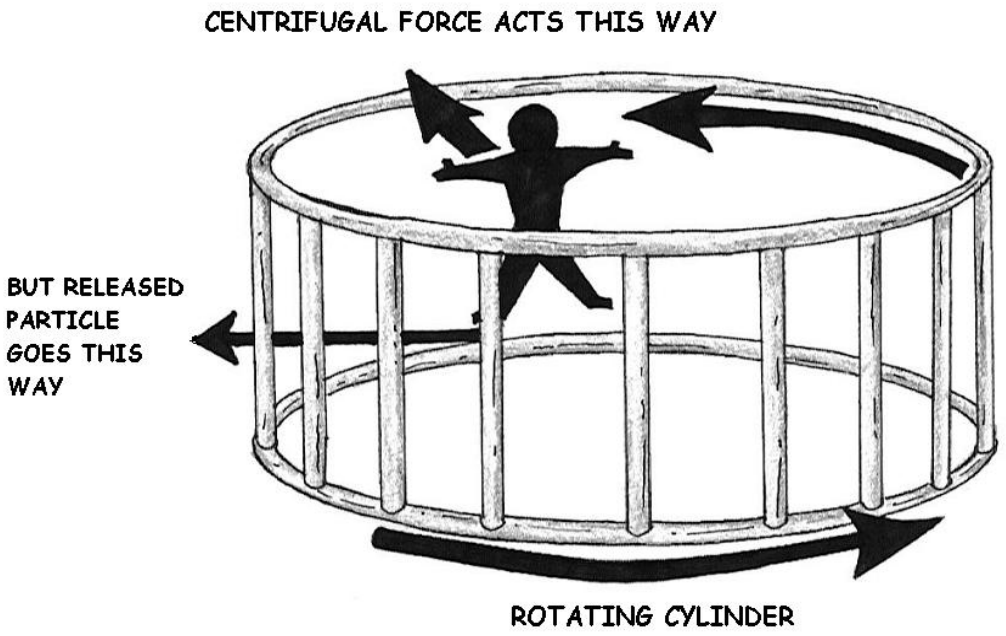


CENTRIFUGAL PUMP

Basic Water Pump

The water pump commonly found in our systems is centrifugal pumps. These pumps work by spinning water around in a circle inside a cylindrical pump housing. The pump makes the water spin by pushing it with an impeller. The blades of this impeller project outward from an axle like the arms of a turnstile and, as the impeller spins, the water spins with it. As the water spins, the pressure near the outer edge of the pump housing becomes much higher than near the center of the impeller.

There are many ways to understand this rise in pressure, and here are two:



First, you can view the water between the impeller blades as an object traveling in a circle. Objects do not naturally travel in a circle--they need an inward force to cause them to accelerate inward as they spin. Without such an inward force, an object will travel in a straight line and will not complete the circle. In a centrifugal pump, that inward force is provided by high-pressure water near the outer edge of the pump housing.

The water at the edge of the pump pushes inward on the water between the impeller blades and makes it possible for that water to travel in a circle. The water pressure at the edge of the turning impeller rises until it is able to keep water circling with the impeller blades.

You can also view the water as an incompressible fluid, one that obeys Bernoulli's equation in the appropriate contexts. As water drifts outward between the impeller blades of the pump, it must move faster and faster because its circular path is getting larger and larger. The impeller blades cause the water to move faster and faster. By the time the water has reached the outer edge of the impeller, it is moving quite fast. However, when the water leaves the impeller and arrives at the outer edge of the cylindrical pump housing, it slows down.

Types of Water Pumps

The most common type of water pumps used for municipal and domestic water supplies are *variable displacement* pumps. A variable displacement pump will produce at different rates relative to the amount of pressure or lift the pump is working against. *Centrifugal* pumps are variable displacement pumps that are by far used the most. The water production well industry almost exclusively uses *Turbine* pumps, which are a type of centrifugal pump.

The turbine pump utilizes *impellers* enclosed in single or multiple *bowls or stages* to lift water by *centrifugal force*. The impellers may be of either a *semi-open or closed type*. Impellers are rotated by the *pump motor*, which provides the horsepower needed to overcome the pumping head. A more thorough discussion of how these and other pumps work is presented later in this section. The size and number of stages, horsepower of the motor and pumping head are the key components related to the pump's lifting capacity.

Vertical turbine pumps are commonly used in groundwater wells. These pumps are driven by a shaft rotated by a motor on the surface. The shaft turns the impellers within the pump housing while the water moves up the column.

This type of pumping system is also called a *line-shaft turbine*. The rotating shaft in a line shaft turbine is actually housed within the column pipe that delivers the water to the surface. The size of the column, impeller, and bowls are selected based on the desired pumping rate and lift requirements.

Column pipe sections can be threaded or coupled together while the drive shaft is coupled and suspended within the column by *spider bearings*. The spider bearings provide both a seal at the column pipe joints and keep the shaft aligned within the column. The water passing through the column pipe serves as the lubricant for the bearings. Some vertical turbines are lubricated by oil rather than water. These pumps are essentially the same as water lubricated units; only the drive shaft is enclosed within an *oil tube*.

Food grade oil is supplied to the tube through a gravity feed system during operation. The oil tube is suspended within the column by *spider flanges,* while the line shaft is supported within the oil tube by *brass or redwood bearings*. A continuous supply of oil lubricates the drive shaft as it proceeds downward through the oil tube.

A small hole located at the top of the pump bow unit allows excess oil to enter the well. This results in the formation of an oil film on the water surface within oil-lubricated wells. Careful operation of oil lubricated turbines is needed to ensure that the pumping levels do not drop enough to allow oil to enter the pump.

Both water and oil lubricated turbine pump units can be driven by electric or fuel powered motors. Most installations use an electric motor that is connected to the drive shaft by a keyway and nut. However, where electricity is not readily available, fuel powered engines may be connected to the drive shaft by a right angle drive gear. Also, both oil and water lubricated systems will have a strainer attached to the intake to prevent sediment from entering the pump.

When the line shaft turbine is turned off, water will flow back down the column, turning the impellers in a reverse direction. A pump and shaft can easily be broken if the motor were to turn on during this process. This is why a *time delay* or *ratchet* assembly is often installed on these motors to either prevent the motor from turning on before reverse rotation stops or simply not allow it to reverse at all.

There are three main types of diaphragm pumps:
In the first type, the diaphragm is sealed with one side in the fluid to be pumped, and the other in air or hydraulic fluid. The diaphragm is flexed, causing the volume of the pump chamber to increase and decrease. A pair of non-return check valves prevents reverse flow of the fluid.

As described above, the second type of diaphragm pump works with volumetric positive displacement, but differs in that the prime mover of the diaphragm is neither oil nor air; but is electro-mechanical, working through a crank or geared motor drive. This method flexes the diaphragm through simple mechanical action, and one side of the diaphragm is open to air. The third type of diaphragm pump has one or more unsealed diaphragms with the fluid to be pumped on both sides. The diaphragm(s) again are flexed, causing the volume to change.

When the volume of a chamber of either type of pump is increased (the diaphragm moving up), the pressure decreases, and fluid is drawn into the chamber. When the chamber pressure later increases from decreased volume (the diaphragm moving down), the fluid previously drawn in is forced out. Finally, the diaphragm moving up once again draws fluid into the chamber, completing the cycle. This action is similar to that of the cylinder in an internal combustion engine.

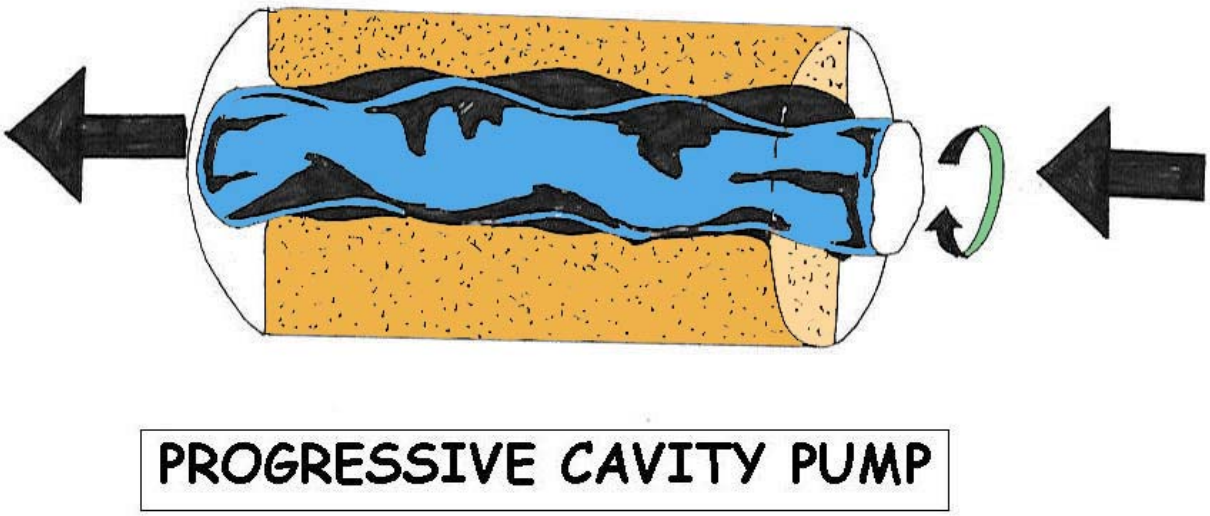
Cavitation
Cavitation is defined as the phenomenon of formation of vapor bubbles of a flowing liquid in a region where the pressure of the liquid falls below its vapor pressure. Cavitation is usually divided into two classes of behavior: inertial (or transient) cavitation and non-inertial cavitation. Inertial cavitation is the process where a void or bubble in a liquid rapidly collapses, producing a shock wave. Such cavitation often occurs in pumps, propellers, impellers, and in the vascular tissues of plants. Non-inertial cavitation is the process in which a bubble in a fluid is forced to oscillate in size or shape due to some form of energy input, such as an acoustic field. Such cavitation is often employed in ultrasonic cleaning baths and can also be observed in pumps, propellers etc.

Cavitation is, in many cases, an undesirable occurrence. In devices such as propellers and pumps, cavitation causes a great deal of noise, damage to components, vibrations, and a loss of efficiency. When the cavitation bubbles collapse, they force liquid energy into very small volumes, thereby creating spots of high temperature and emitting shock waves, the latter of which are a source of noise. The noise created by cavitation is a particular problem for military submarines, as it increases the chances of being detected by passive sonar. Although the collapse of a cavity is a relatively low-energy event, highly localized collapses can erode metals, such as steel, over time. The pitting caused by the collapse of cavities produces great wear on components and can dramatically shorten a propeller's or pump's lifetime.

After a surface is initially affected by cavitation, it tends to erode at an accelerating pace. The cavitation pits increase the turbulence of the fluid flow and create crevasses that act as nucleation sites for additional cavitation bubbles. The pits also increase the component's surface area and leave behind residual stresses. This makes the surface more prone to stress corrosion.

Impeller
An impeller is a rotating component of a centrifugal pump, usually made of iron, steel, aluminum or plastic, which transfers energy from the motor that drives the pump to the fluid being pumped by accelerating the fluid outwards from the center of rotation. The velocity achieved by the impeller transfers into pressure when the outward movement of the fluid is confined by the pump casing. Impellers are usually short cylinders with an open inlet (called an eye) to accept incoming fluid, vanes to push the fluid radically, and a splined center to accept a driveshaft.

Progressing Cavity Pump



In this type of pump, components referred to as a rotor and an elastic stator provide the mechanical action used to force liquid from the suction side to the discharge side of the pump. As the rotor turns within the stator, cavities are formed which progress from the suction to the discharge end of the pump, conveying the pumped material. The continuous seal between the rotor and the stator helices keeps the fluid moving steadily at a fixed flow rate proportional to the pump's rotational speed. Progressing cavity pumps are used to pump material very high in solids content. The progressive cavity pump must never be run dry, because the friction between the rotor and stator will quickly damage the pump.

More on the Progressive Cavity Pump

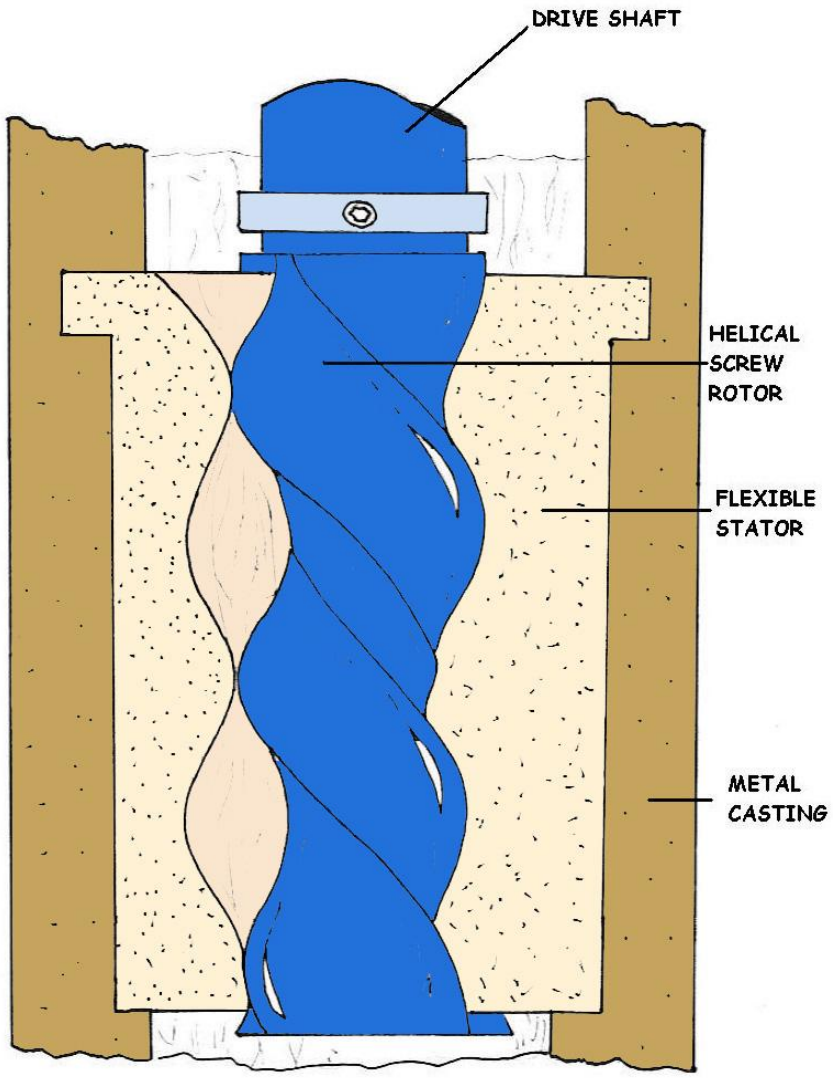
A progressive cavity pump is also known as a progressing cavity pump, eccentric screw pump, or even just cavity pump, and as is common in engineering generally, these pumps can often be referred to by using a generalized trademark. Hence, names can vary from industry to industry and even regionally; examples include: Mono pump, Moyno pump, Mohno pump, and Nemo pump. This type of pump transfers fluid by means of the progress, through the pump, of a sequence of small, fixed shape, discrete cavities, as its rotor is turned. This leads to the volumetric flow rate being proportional to the rotation rate (bi-directionally) and to low levels of shearing being applied to the pumped fluid. Hence, these pumps have application in fluid metering and pumping of viscous or shear sensitive materials. It should be noted that the cavities taper down toward their ends and overlap with their neighbors, so that, in general, no flow pulsing is caused by the arrival of cavities at the outlet, other than that caused by compression of the fluid or pump components.

The principle of this pumping technique is frequently misunderstood; often it is believed to occur due to a dynamic effect caused by drag, or friction against the moving teeth of the screw rotor. However, in reality it is due to sealed cavities, like a piston pump, and so has similar operational characteristics, such as being able to pump at extremely low rates, even to high pressure, revealing the effect to be purely positive displacement. The mechanical layout that causes the cavities to, uniquely, be of fixed dimensions as they move through the pump, is hard to visualize (it's essentially 3D nature renders diagrams quite ineffective for explanation), but it is accomplished by the preservation in shape of the gap formed between a helical shaft and a two

start, twice the wavelength and double the diameter, helical hole, as the shaft is "rolled" around the inside surface of the hole. The motion of the rotor being the same as the smaller gears of a planetary gears system. This form of motion gives rise to the curves called Hypocycloids.

In order to produce a seal between cavities, the rotor requires a circular cross-section and the stator an oval one. The rotor so takes a form similar to a corkscrew, and this, combined with the off-center rotary motion, leads to the name; ***Eccentric screw pump***.

Different rotor shapes and rotor/stator pitch ratios exist, but are specialized in that they don't generally allow complete sealing, so reducing low speed pressure and flow rate linearity, but improving actual flow rates, for a given pump size, and/or the pump's solids handling ability.



PROGRESSIVE CAVITY PUMP

At a high enough pressure the sliding seals between cavities will leak some fluid rather than pumping it, so when pumping against high pressures a longer pump with more cavities is more effective, since each seal has only to deal with the pressure difference between adjacent cavities. Pumps with between two and a dozen or so cavities exist.

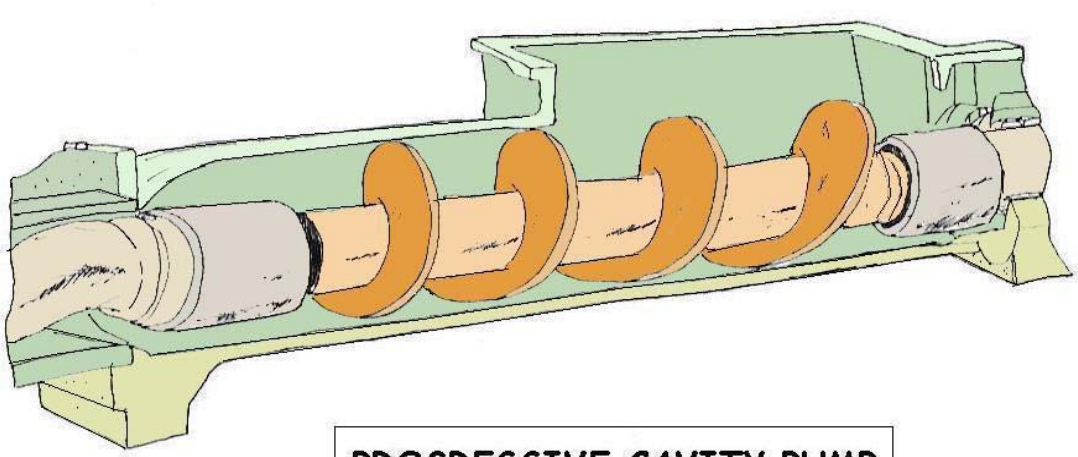
In operation, progressive cavity pumps are fundamentally fixed flow rate pumps, like piston pumps and peristaltic pumps. This type of pump needs a fundamentally different understanding to the types of pumps to which people are more commonly first introduced, namely ones that can be thought of as generating a pressure. This can lead to the mistaken assumption that all pumps can have their flow rates adjusted by using a valve attached to their outlet, but with this type of pump this assumption is a problem, since such a valve will have practically no effect on the flow rate and completely closing it will involve very high, probably damaging, pressures being generated. In order to prevent this, pumps are often fitted with cut-off pressure switches, burst disks (deliberately weak and easily replaced points), or a bypass pipe that allows a variable amount of a fluid to return to the inlet. With a bypass fitted, a fixed flow rate pump is effectively converted to a fixed pressure one.

At the points where the rotor touches the stator, the surfaces are generally traveling transversely, so small areas of sliding contact occur, these areas need to be lubricated by the fluid being pumped (Hydrodynamic lubrication), this can mean that more torque is required for starting, and if allowed to operate without fluid, called **'run dry'**, rapid deterioration of the stator can result.

While progressive cavity pumps offer long life and reliable service transporting thick or lumpy fluids, abrasive fluids will significantly shorten the life of the stator. However, slurries (particulates in a medium) can be pumped reliably, as long as the medium is viscous enough to maintain a lubrication layer around the particles and so provide protection to the stator.

Specific designs involve the rotor of the pump being made of a steel, coated in a smooth hard surface, normally chromium, with the body (the stator) made of a molded elastomer inside a metal tube body. The Elastomer core of the stator forms the required complex cavities. The rotor is held against the inside surface of the stator by angled link arms, bearings (which have to be within the fluid) allowing it to roll around the inner surface (un-driven). Elastomer is used for the stator to simplify the creation of the complex internal shape, created by means of casting, and also improves the quality and longevity of the seals by progressively swelling due to absorption of water and/or other common constituents of pumped fluids. Elastomer/pumped fluid compatibility will thus need to be taken into account.

Two common designs of stator are the "***Equal-walled***" and the "***Unequal walled***". The latter, having greater elastomer wall thickness at the peaks allows larger-sized solids to pass through because of its increased ability to distort under pressure.



PROGRESSIVE CAVITY PUMP

Key Pump Words

NPSH: Net positive suction head - related to how much suction lift a pump can achieve by creating a partial vacuum. Atmospheric pressure then pushes liquid into the pump. A method of calculating if the pump will work or not.

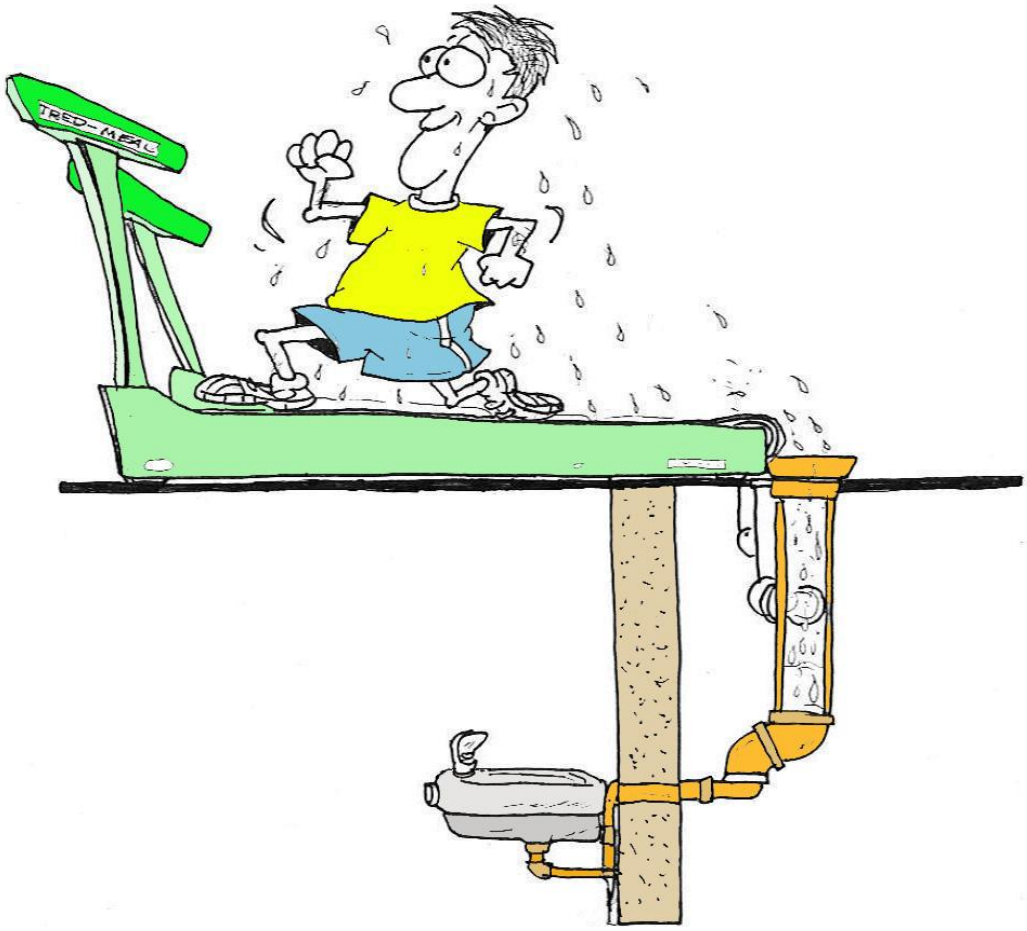
S.G.: Specific gravity. Weight of liquid in comparison to water at approx. 20 deg c (SG = 1).

Specific Speed: A number which is the function of pump flow, head, efficiency etc. Not used in day to day pump selection, but very useful, as pumps with similar specific speed will have similar shaped curves, similar efficiency / NPSH / solids handling characteristics.

Vapor Pressure: If the vapor pressure of a liquid is greater than the surrounding air pressure, the liquid will boil.

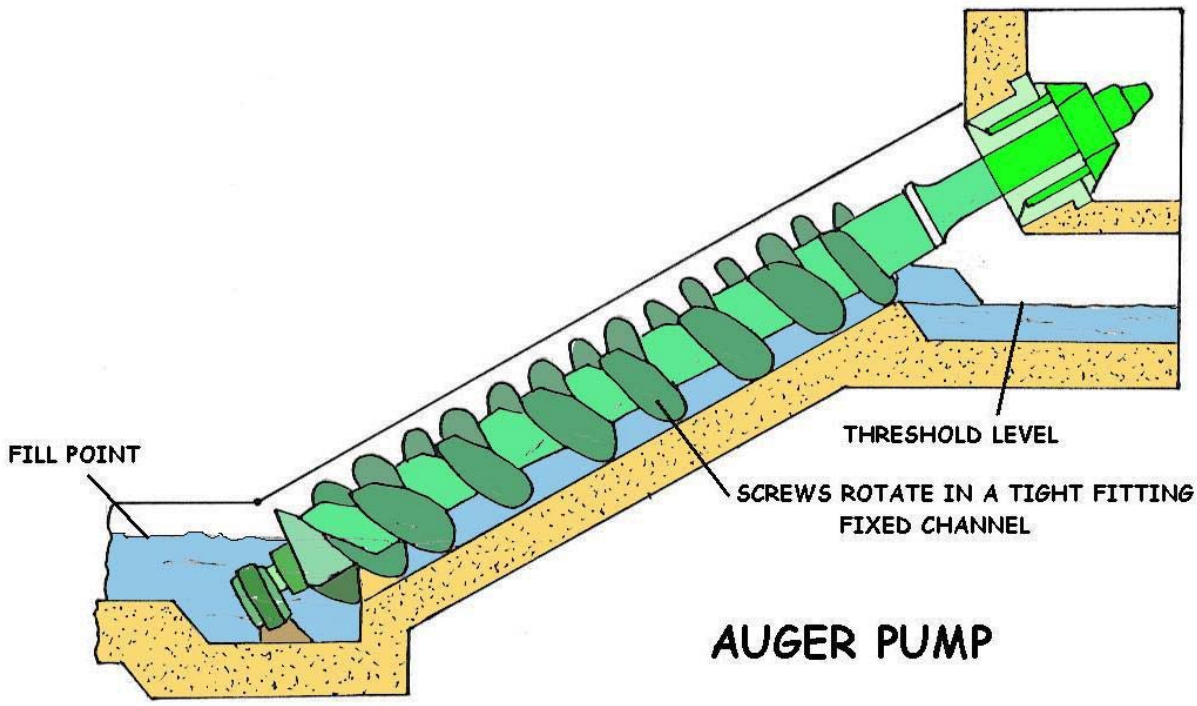
Viscosity: A measure of a liquid's resistance to flow. i.e.: how thick it is. The viscosity determines the type of pump used, the speed it can run at, and with gear pumps, the internal clearances required.

Friction Loss: The amount of pressure / head required to 'force' liquid through pipe and fittings.



Screw or Auger Pump

The Archimedes' screw, Archimedean screw, or screwpump is a machine historically used for transferring water from a low-lying body of water into irrigation ditches. It was one of several inventions and discoveries traditionally attributed to Archimedes in the 3rd century BC.



The machine consists of a screw inside a hollow pipe. Some attribute its invention to Archimedes in the 3rd century BC, while others attribute it to Nebuchadnezzar II in the 7th century BC. A screw can be thought of as an inclined plane (another simple machine) wrapped around a cylinder.

The screw is turned (usually by a windmill or by manual labor). As the bottom end of the tube turns, it scoops up a volume of water. This amount of water will slide up in the spiral tube as the shaft is turned, until it finally pours out from the top of the tube and feeds the irrigation system.

The contact surface between the screw and the pipe does not need to be perfectly water-tight because of the relatively large amount of water being scooped at each turn with respect to the angular speed of the screw. Also, water leaking from the top section of the screw leaks into the previous one and so on. So a sort of equilibrium is achieved while using the machine, thus preventing a decrease in efficiency.

The "screw" does not necessarily need to turn inside the casing, but can be allowed to turn with it in one piece. A screw could be sealed with pitch or some other adhesive to its casing, or, cast as a single piece in bronze, as some researchers have postulated as being the devices used to irrigate Nebuchadnezzar II's Hanging Gardens of Babylon. Depictions of Greek and Roman water screws show the screws being powered by a human treading on the outer casing to turn the entire apparatus as one piece, which would require that the casing be rigidly attached to the screw.

In this type of pump, a large screw provides the mechanical action to move the liquid from the suction side to the discharge side of the pump. Here are some typical characteristics of screw pumps:

- Most screw pumps rotate in the 30 to 60 rpm range, although some screw pumps are faster.
- The slope of the screw is normally either 30° or 38°.

The maximum lift for the larger diameter pumps is about 30 feet. The smaller diameter pumps have lower lift capabilities.

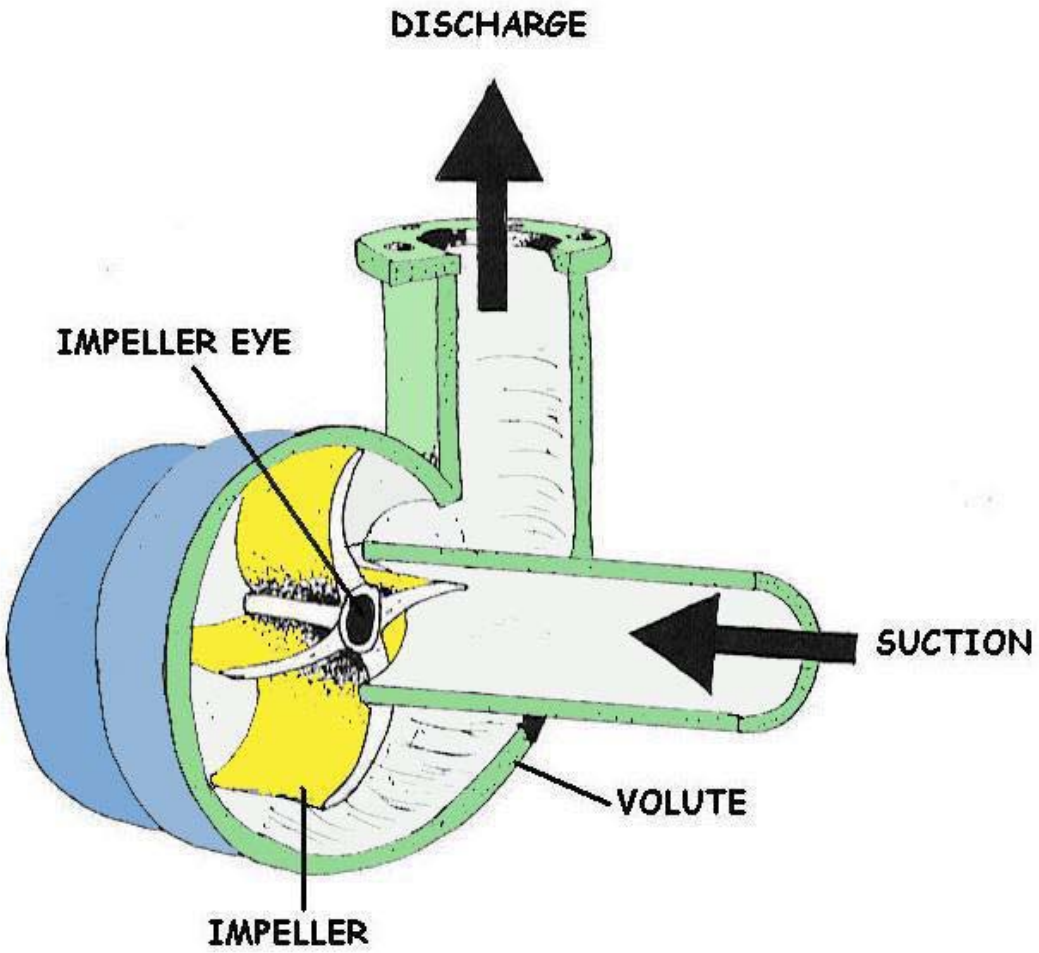
Centrifugal Pump

By definition, a centrifugal pump is a machine. More specifically, it is a machine that imparts energy to a fluid. This energy infusion can cause a liquid to flow, rise to a higher level, or both.

The centrifugal pump is an extremely simple machine. It is a member of a family known as rotary machines and consists of two basic parts: 1) the rotary element or impeller and 2) the stationary element or casing (volute). The figure at the bottom of the page is a cross section of a centrifugal pump and shows the two basic parts.

In operation, a centrifugal pump "**slings**" liquid out of the impeller via centrifugal force. One fact that must always be remembered: A pump does not create pressure, it only provides flow. Pressure is just an indication of the amount of resistance to flow.

Centrifugal pumps may be classified in several ways. For example, they may be either **SINGLE STAGE** or **MULTI-STAGE.** A single-stage pump has only one impeller. A multi-stage pump has two or more impellers housed together in one casing.



As a rule, each impeller acts separately, discharging to the suction of the next stage impeller. This arrangement is called series staging. Centrifugal pumps are also classified as HORIZONTAL or VERTICAL, depending upon the position of the pump shaft.

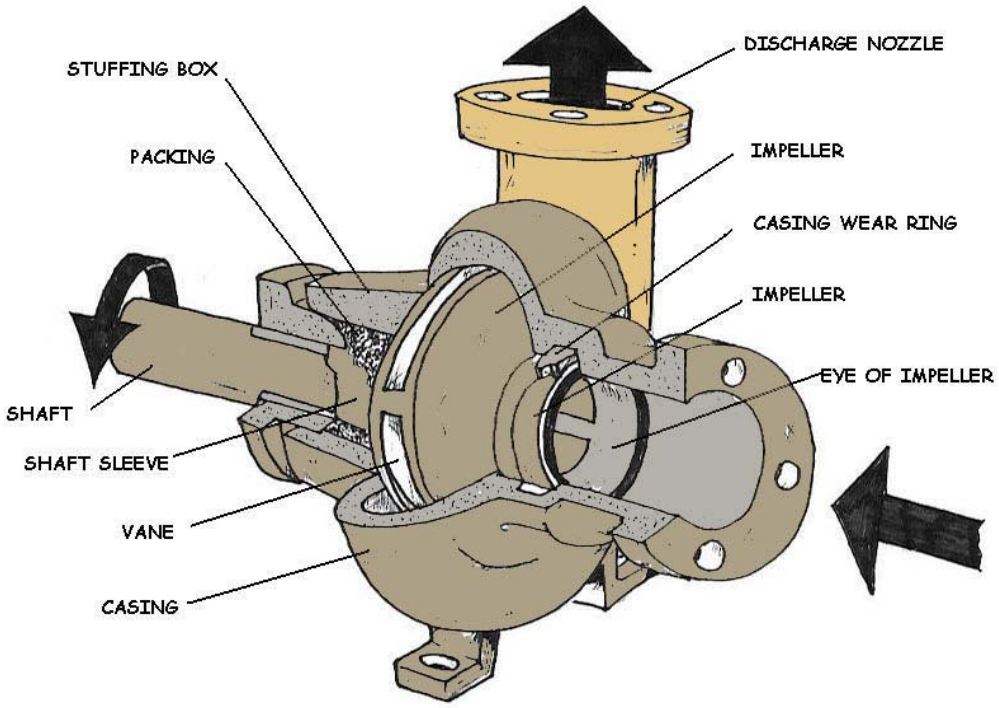
The impellers used on centrifugal pumps may be classified as SINGLE SUCTION or DOUBLE SUCTION. The single-suction impeller allows liquid to enter the eye from one side only. The double-suction impeller allows liquid to enter the eye from two directions.

Impellers are also classified as CLOSED or OPEN. Closed impellers have side walls that extend from the eye to the outer edge of the vane tips. Open impellers do not have these side walls. Some small pumps with single-suction impellers have only a casing wearing ring and no impeller ring. In this type of pump, the casing wearing ring is fitted into the end plate. Recirculation lines are installed on some centrifugal pumps to prevent the pumps from overheating and becoming vapor bound, in case the discharge is entirely shut off or the flow of fluid is stopped for extended periods.

Seal piping is installed to cool the shaft and the packing, to lubricate the packing, and to seal the rotating joint between the shaft and the packing against air leakage. A lantern ring spacer is inserted between the rings of the packing in the stuffing box. Seal piping leads the liquid from the discharge side of the pump to the annular space formed by the lantern ring. The web of the ring is perforated so that the water can flow in either direction along the shaft (between the shaft and the packing).

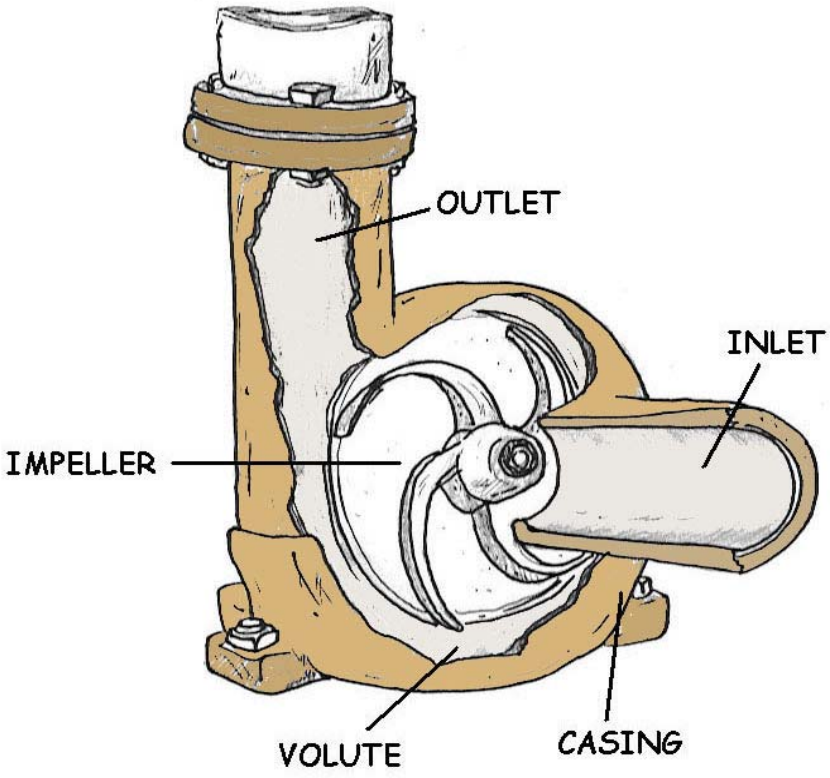
Water flinger rings are fitted on the shaft between the packing gland and the pump bearing housing. These flingers prevent water in the stuffing box from flowing along the shaft and entering the bearing housing.

Let's look at the components of the centrifugal pump.

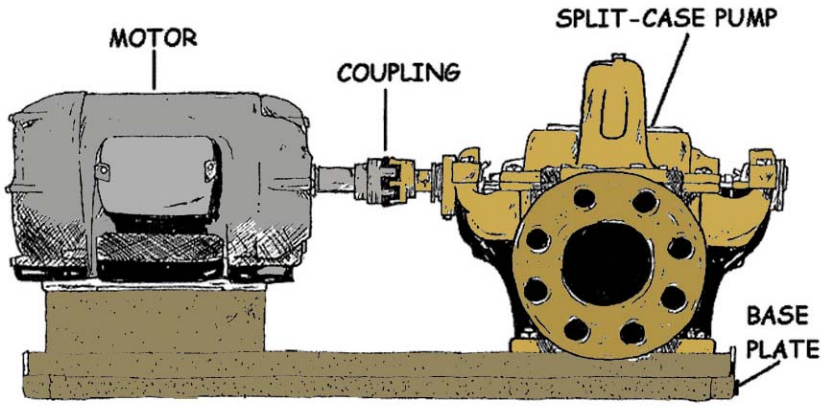


Centrifugal Pump

As the impeller rotates, it sucks the liquid into the center of the pump and throws it out under pressure through the outlet. The casing that houses the impeller is referred to as the volute, the impeller fits on the shaft inside. The volute has an inlet and outlet that carries the water as shown above.



These pictures illustrate the components that are common to most pump assemblies.



NPSH - Net Positive Suction Head
If you accept that a pump creates a partial vacuum and atmospheric pressure forces water into the suction of the pump, then you will find NPSH a simple concept.

NPSH (a) is the Net Positive Suction Head Available, which is calculated as follows:

NPSH (a) = p + s - v - f

Where:
'p'= atmospheric pressure,
's'= static suction (If liquid is below pump, it is shown as a negative value)
'v'= liquid vapor pressure
'f'= friction loss

NPSH (a) must exceed NPSH(r) to allow pump operation without cavitation. (It is advisable to allow approximately 1 meter difference for most installations.) The other important fact to remember is that water will boil at much less than 100 degrees C^{O} if the pressure acting on it is less than its vapor pressure, i.e. water at 95 degrees C is just hot water at sea level, but at 1500m above sea level it is boiling water and vapor.

The vapor pressure of water at 95 degrees C is 84.53 kPa, there was enough atmospheric pressure at sea level to contain the vapor, but once the atmospheric pressure dropped at the higher elevation, the vapor was able to escape. This is why vapor pressure is always considered in NPSH calculations when temperatures exceed 30 to 40 deg C.

NPSH(r) is the Net Positive Suction Head Required by the pump, which is read from the pump performance curve. (Think of NPSH(r) as friction loss caused by the entry to the pump suction.)

Affinity Laws

The Centrifugal Pump is a very capable and flexible machine. Because of this it is unnecessary to design a separate pump for each job. The performance of a centrifugal pump can be varied by changing the impeller diameter or its rotational speed. Either change produces approximately the same results. Reducing impeller diameter is probably the most common change and is usually the most economical. The speed can be altered by changing pulley diameters or by changing the speed of the driver. In some cases both speed and impeller diameter are changed to obtain the desired results.

When the driven speed or impeller diameter of a centrifugal pump changes, operation of the pump changes in accordance with three fundamental laws. These laws are known as the "Laws of Affinity". They state that:
1) Capacity varies directly as the change in speed
2) Head varies as the square of the change in speed
3) Brake horsepower varies as the cube of the change in speed
If, for example, the pump speed were doubled:
1) Capacity will double
2) Head will increase by a factor of 4 (2 to the second power)
3) Brake horsepower will increase by a factor of 8 (2 to the third power)
These principles apply regardless of the direction (up or down) of the speed or change in diameter.
Consider the following example. A pump operating at 1750 RPM, delivers 210 GPM at 75' TDH, and requires 5.2 brake horsepower. What will happen if the speed is increased to 2000 RPM? First we find the speed ratio.

Speed Ratio = 2000/1750 = 1.14

From the laws of Affinity:
1) Capacity varies directly or:
1.14 X 210 GPM = 240 GPM
2) Head varies as the square or:

1.14 X 1.14 X 75 = 97.5' TDH
3) BHP varies as the cube or:

1.14 X 1.14 X 1.14 X 5.2 = 7.72 BHP
Theoretically, the efficiency is the same for both conditions. By calculating several points a new curve can be drawn.

Whether it be a speed change or change in impeller diameter, the Laws of Affinity give results that are approximate. The discrepancy between the calculated values and the actual values obtained in test are due to hydraulic efficiency changes that result from the modification. The Laws of Affinity give reasonably close results when the changes are not more than 50% of the original speed or 15% of the original diameter.

Suction conditions are some of the most important factors affecting centrifugal pump operation. If they are ignored during the design or installation stages of an application, they will probably come back to haunt you.

Suction Lift
A pump cannot pull or "suck" a liquid up its suction pipe because liquids do not exhibit tensile strength. Therefore, they cannot transmit tension or be pulled. When a pump creates a suction, it is simply reducing local pressure by creating a partial vacuum. Atmospheric or some other external pressure acting on the surface of the liquid pushes the liquid up the suction pipe into the pump.

Atmospheric pressure at sea level is called absolute pressure (PSIA) because it is a measurement using absolute zero (a perfect vacuum) as a base. If pressure is measured using atmospheric pressure as a base it is called gauge pressure (PSIG or simply PSI).

Atmospheric pressure, as measured at sea level, is 14.7 PSIA. In feet of head it is:
Head = PSI X 2.31 / Specific Gravity

For Water it is:
Head = 14.7 X 2.31 / 1.0 = 34 Ft

Thus, 34 feet is the theoretical maximum suction lift for a pump pumping cold water at sea level. No pump can attain a suction lift of 34 ft; however, well designed ones can reach 25 ft quite easily.

You will note, from the equation above, that specific gravity can have a major effect on suction lift. For example, the theoretical maximum lift for brine (Specific Gravity = 1.2) at sea level is 28 ft.. The realistic maximum is around 20ft. Remember to always factor in specific gravity if the liquid being pumped is anything but clear, cold (68 degrees F) water. In addition to pump design and suction piping, there are two physical properties of the liquid being pumped that affect suction lift.

1) Maximum suction lift is dependent upon the pressure applied to the surface of the liquid at the suction source. Maximum suction lift decreases as pressure decreases.

2) 2) Maximum suction lift is dependent upon the vapor pressure of the liquid being pumped. The vapor pressure of a liquid is the pressure necessary to keep the liquid from vaporizing (boiling) at a given temperature. Vapor pressure increases as liquid temperature increases. Maximum suction lift decreases as vapor pressure rises.

It follows then, that the maximum suction lift of a centrifugal pump varies inversely with altitude. Conversely, maximum suction lift will increase as the external pressure on its source increases (for example: a closed pressure vessel).

Cavitation - Two Main Causes:
A. NPSH (r) EXCEEDS NPSH (a)
Due to low pressure the water vaporizes (boils), and higher pressure implodes into the vapor bubbles as they pass through the pump, causing reduced performance and potentially major damage.
B. Suction or discharge recirculation. The pump is designed for a certain flow range, if there is not enough or too much flow going through the pump, the resulting turbulence and vortexes can reduce performance and damage the pump.

Affinity Laws - Centrifugal Pumps
If the speed or impeller diameter of a pump changes, we can calculate the resulting performance change using:

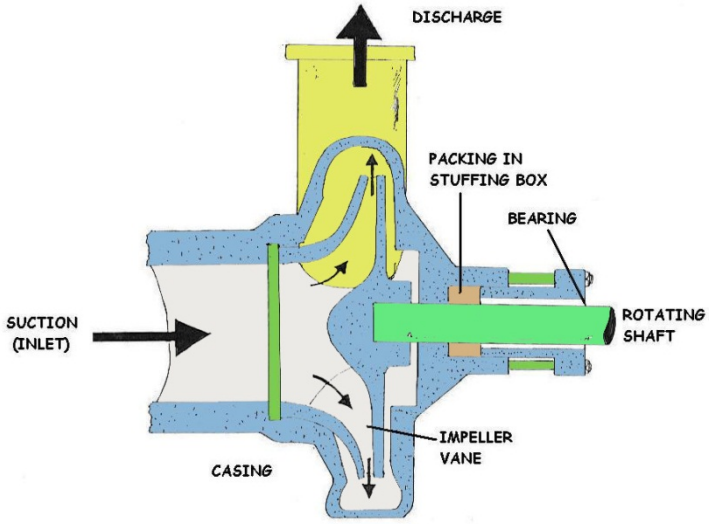
Affinity laws
a. The flow changes proportionally to speed
i.e.: double the speed / double the flow

b. The pressure changes by the square of the difference
i.e.: double the speed / multiply the pressure by 4

c. The power changes by the cube of the difference
i.e.: double the speed / multiply the power by 8

Notes:
1. These laws apply to operating points at the same efficiency.

2. Variations in impeller diameter greater than 10% are hard to predict due to the change in relationship between the impeller and the casing. For rough calculations you can adjust a duty point or performance curve to suit a different speed. NPSH (r) is affected by speed / impeller diameter change = ***DANGER !***



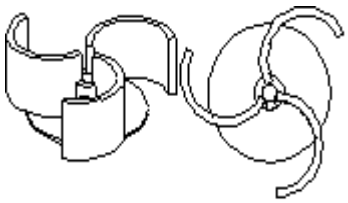
Pump Casing

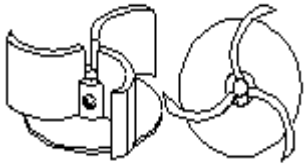
There are many variations of centrifugal pumps. The most common type is an end suction pump. Another type of pump used is the split case. There are many variations of split case, such as; two-stage, single suction, and double suction. Most of these pumps are horizontal.

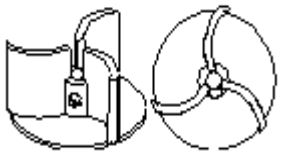
There are variations of vertical centrifugal pumps. The line shaft turbine is really a multistage centrifugal pump.

Impeller
In most centrifugal pumps, the impeller looks like a number of cupped vanes on blades mounted on a disc or shaft. Notice in the picture below how the vanes of the impeller force the water into the outlet of the pipe.

The shape of the vanes of the impeller is important. As the water is being thrown out of the pump, this means you can run centrifugal pumps with the discharged valve closed for a **SHORT** period of time. Remember the motor sends energy along the shaft, and if the water is in the volute too long it will heat up and create steam. Not good!







Impellers are designed in various ways. We will look at:

- Closed impellers
- Semi-open impellers
- Opened impellers, and
- Recessed impellers

The impellers all cause a flow from the eye of the impeller to the outside of the impeller. These impellers cause what is called **radial flow**, and they can be referred to as radial flow impellers.

The **critical distance** of the impeller and how it is installed in the casing will determine if it is high volume / low pressure or the type of liquid that could be pumped.

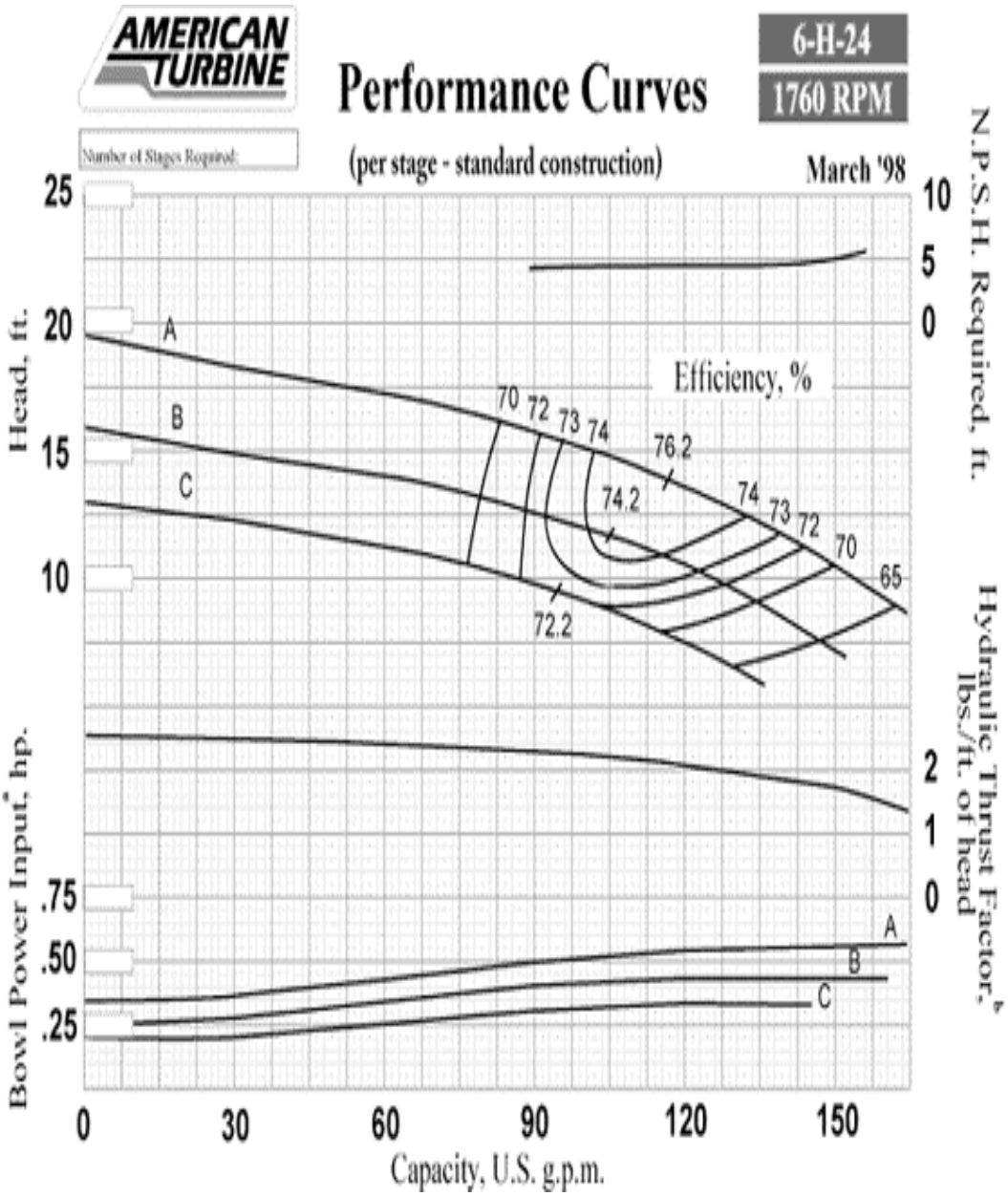
Axial flow impellers look like a propeller and create a flow that is parallel to the shaft.

PNUEMATIC SUBMERSIBLE PUMP

Pump Performance and Curves

Lets looks at the big picture. Before you make that purchase of the pump and motor you need to know the basics such as:

- Total dynamic head, the travel distance
- Capacity, how much water you need to provide
- Efficiency, help determine the impeller size
- HP, how many squirrels you need
- RPM, how fast the squirrels run



Motor and Pump Calculations

The centrifugal pump pumps the difference between the suction and the discharge heads. There are three kinds of discharge head:

- **Static head.** The height we are pumping to, or the height to the discharge piping outlet that is filling the tank from the top. Note: that if you are filling the tank from the bottom, the static head will be constantly changing.
- **Pressure head.** If we are pumping to a pressurized vessel (like a boiler) we must convert the pressure units (psi. or Kg.) to head units (feet or meters).
- **System or dynamic head.** Caused by friction in the pipes, fittings, and system components. We get this number by making the calculations from published charts.

Suction head is measured the same way.

- If the liquid level is above the pump center line, that level is a positive suction head. If the pump is lifting a liquid level from below its center line, it is a negative suction head.
- If the pump is pumping liquid from a pressurized vessel, you must convert this pressure to a positive suction head. A vacuum in the tank would be converted to a negative suction head.
- Friction in the pipes, fittings, and associated hardware is a negative suction head.
- Negative suction heads are added to the pump discharge head, positive suctions heads are subtracted from the pump discharge head.

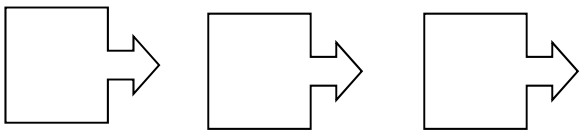
Total Dynamic Head (TDH) is the total height that a fluid is to be pumped, taking into account friction losses in the pipe.

TDH = Static Lift + Static Height + Friction Loss

where:
Static Lift is the height the water will rise before arriving at the pump (also known as the 'suction head').

Static Height is the maximum height reached by the pipe after the pump (also known as the 'discharge head').

Friction Loss is the head equivalent to the energy losses due to viscose drag of fluid flowing in the pipe (both on the suction and discharge sides of the pump). It is calculated via a formula or a chart, taking into account the pipe diameter and roughness and the fluid flow rate, density, and viscosity.



Motor hp **Brake hp** **Water hp**

Horsepower

Work involves the operation of force over a specific distance. The rate of doing work is called power.
The rate in which a horse could work was determined to be about 550 ft-lbs/sec or 33,000 ft-lbs/min.

1 hp = 33,000 ft-lbs/min

Motor Horsepower (mhp)

1 hp = 746 watts or .746 Kilowatts

MHP refers to the horsepower supplied in the form of electrical current. The efficiency of most motors range from 80-95%. (Manufactures will list efficiency %)

Brake Horsepower (bhp)

$$\text{Brake hp} = \frac{\text{Water hp}}{\text{Pump Efficiency}}$$

BHP refers to the horsepower supplied to the pump from the motor. As the power moves through the pump, additional horsepower is lost, resulting from slippage and friction of the shaft and other factors.

Water Horsepower

$$\text{Water hp} = \frac{(\text{flow gpm})(\text{total hd})}{3960}$$

Water horsepower refers to the actual horse power available to pump the water.

Horsepower and Specific Gravity

The specific gravity of a liquid is an indication of its density or weight compared to water. The difference in specific gravity, include it when calculating ft-lbs/min pumping requirements.

$$\frac{(\text{ft})(\text{lbs/min})(\text{sp.gr.})}{33{,}000 \text{ ft-lbs/min/hp}} = \text{whp}$$

MHP and Kilowatt requirements

$$1 \text{ hp} = 0.746 \text{ kW} \quad \text{or} \quad \frac{(\text{hp})\ (746 \text{ watts/hp})}{1000 \text{ watts/kW}}$$

Well Calculations

1. Well drawdown

Drawdown ft = Pumping water level, ft - Static water level, ft

2. Well yield

$$\text{Well yield, gpm} = \frac{\text{Flow, gallons}}{\text{Duration of test, min}}$$

3. Specific yield

$$\text{Specific yield, gpm/ft} = \frac{\text{Well yield, gpm}}{\text{Drawdown, ft}}$$

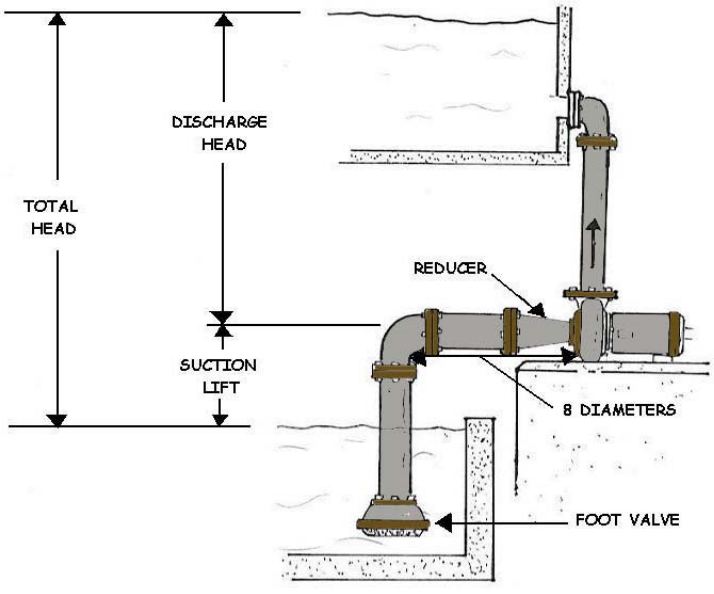
4. Deep well turbine pump calculations.

Discharge head, ft = (pressure measured) (2.31 ft/psi)

Field head, ft = pumping water + discharge head, ft

Bowl head, ft = field head + column friction

1 psi = 2.31 feet of head
1 foot of head = .433 psi



Example 1

A centrifugal pump is located at an elevation of 722 ft. This pump is used to move water from reservoir **A** to reservoir **B**. The water level in reservoir **A** is 742 ft and the water level in reservoir **B** is 927 ft. Based on these conditions answer the following questions:

1. **If the pump is not running and pressure gauges are installed on the suction and discharge lines, what pressures would the gauges read?**

   Suction side:

   Discharge side:

2. **How can you tell if this is a suction head condition?**

3. **Calculate the following head measurements:**

SSH:

SDH:

TSH:

4. **Convert the pressure gauge readings to feet:**

6 psi:

48 psi:

110 psi:

5. **Calculate the following head in feet to psi:**

20 ft:

205 ft:

185 ft:

Motor, Coupling, and Bearing Section

We will now refer to the motor, coupling, and bearings. The power source of the pump is usually an electric motor. The motor is connected by a coupling to the pump shaft. The purpose of the bearings is to hold the shaft firmly in place, yet allow it to rotate. The bearing house supports the bearings and provides a reservoir for the lubricant. An impeller is connected to the shaft. The pump assembly can be a vertical or horizontal set-up; the components for both are basically the same.

Motors

The purpose of this discussion on pump motors is to identify and describe the main types of motors, starters, enclosures, and motor controls, as well as to provide you with some basic maintenance and troubleshooting information. Although pumps could be driven by diesel or gasoline engines, pumps driven by electric motors are commonly used in our industry.

There are two general categories of electric motors:

- D-C motors, or direct current
- A-C motors, or alternating current

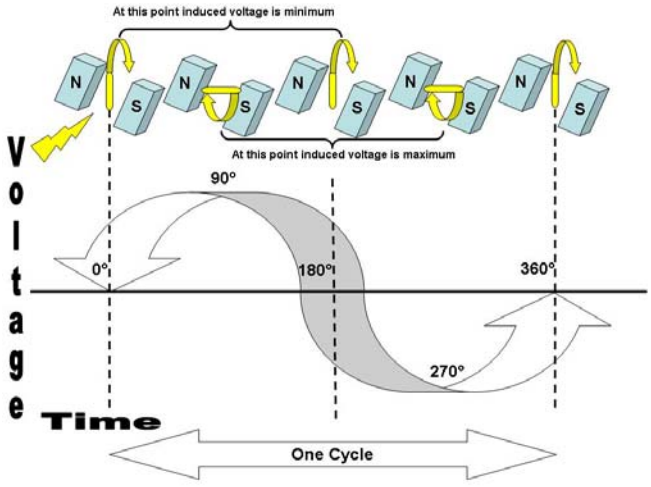
You can expect most motors at facilities to be A-C type.

D-C Motors

The important characteristic of the D-C motor is that its speed will vary with the amount of current used. There are many different kinds of D-C motors, depending on how they are wound and their speed/torque characteristics.

A-C Motors

There are a number of different types of alternating current motors, such as Synchronous, Induction, wound rotor, and squirrel cage. The synchronous type of A-C motor requires complex control equipment, since they use a combination of A-C and D-C. This also means that the synchronous type of A-C motor is used in large horsepower sizes, usually above 250 HP. The induction type motor uses only alternating current. The squirrel cage motor provides a relatively constant speed. The wound rotor type could be used as a variable speed motor.



Define the Following Terms:

Voltage:

EMF:

Power:

Current:

Resistance:

Conductor:

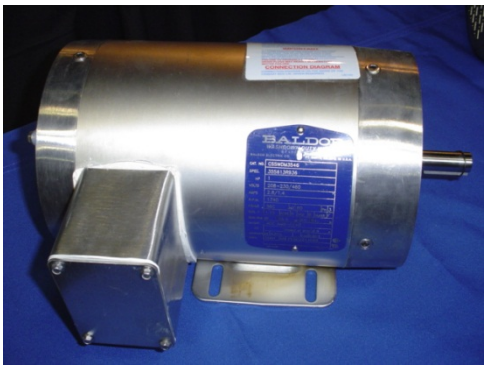
Phase:

Single Phase:

Three Phase:

Hertz:

Motor Starters



All electric motors, except very small ones such as chemical feed pumps, are equipped with starters, either full voltage or reduced voltage. This is because motors draw a much higher current when they are starting and gaining speed. The purpose of the reduced voltage starter is to prevent the load from coming on until the amperage is low enough. How do you think keeping the discharge valve closed on a centrifugal pump could reduce the start -up load?

Motor Enclosures

Depending on the application, motors may need special protection. Some motors are referred to as open motors. They allow air to pass through to remove heat generated when current passes through the windings. Other motors use specific enclosures for special environments or safety protection.

Can you think of any locations within your facility that requires special enclosures?

Two Types of Totally Enclosed Motors Commonly Used are:

- **TENV**, or totally enclosed non-ventilated motor
- **TEFC**, or totally enclosed fan cooled motor

Totally enclosed motors include dust-proof, water-proof and explosion-proof motors. An explosion proof enclosure must be provided on any motor where dangerous gases might accumulate.

Motor Controls
All pump motors are provided with some method of control, typically a combination of manual and automatic. Manual pump controls can be located at the central control panel at the pump or at the suction or discharge points of the liquid being pumped.

There are a number of ways in which automatic control of a pump motor can be regulated:

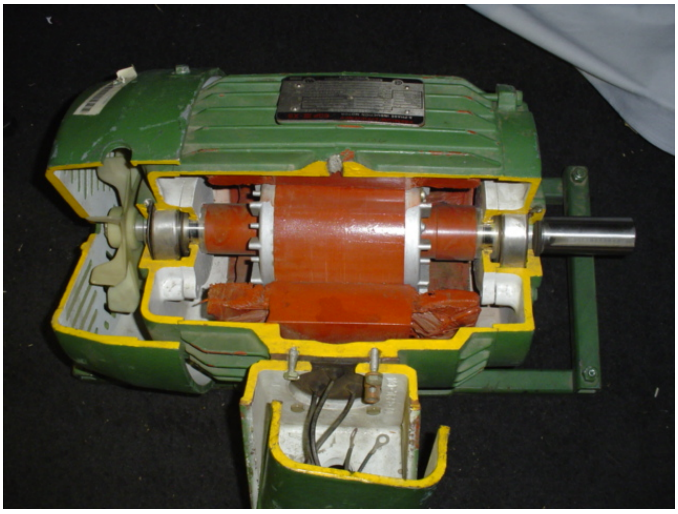
- Pressure and vacuum sensors
- Preset time intervals
- Flow sensors
- Level sensors

Two typical level sensors are the float sensor and the bubble regulator. The float sensor is pear-shaped and hangs in the wet well. As the height increases, the float tilts, and the mercury in the glass tube flows toward the end of the tube that has two wires attached to it. When the mercury covers the wires, it closes the circuit.

A low pressure air supply is allowed to escape from a bubbler pipe in the wet well. The back-pressure on the air supply will vary with the liquid level over the pipe. Sensitive air pressure switches will detect this change and use this information to control pump operation.

Motor Maintenance
Motors should be kept clean, free of moisture, and lubricated properly. Dirt, dust, and grime will plug the ventilating spaces and can actually form an insulating layer over the metal surface of the motor.

What condition would occur if the ventilation becomes blocked?



Moisture

Moisture harms the insulation on the windings to the point where they may no longer provide the required insulation for the voltage applied to the motor. In addition, moisture on windings tend to absorb acid and alkali fumes, causing damage to both insulation and metals. To reduce problems caused by moisture, the most suitable motor enclosure for the existing environment will normally be used. It is recommended to run stand by motors to dry up any condensation which accumulates in the motor.

Motor Lubrication
Friction will cause wear in all moving parts, and lubrication is needed to reduce this friction. It is very important that all your manufacturer's recommended lubrication procedures are strictly followed. You have to be careful not to add too much grease or oil, as this could cause more friction and generate heat.

To grease the motor bearings, this is the usual approach:

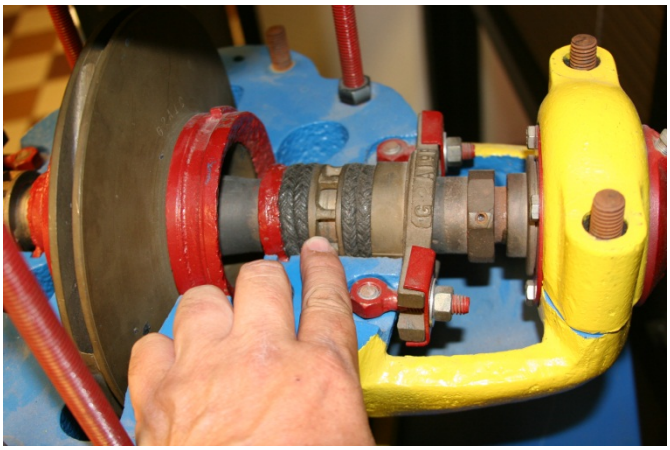
1. Remove the protective plugs and caps from the grease inlet and relief holes.
2. Pump grease in until fresh starts coming from the relief hole.

If fresh grease does not come out of the relief hole, this could mean that the grease has been pumped into the motor windings. The motor must then be taken apart and cleaned by a qualified service representative.

To change the oil in an oil lubricated motor, this is the usual approach:

1. Remove all plugs and let the oil drain.
2. Check for metal shearing.
3. Replace the oil drain.
4. Add new oil until it is up to the oil level plug.
5. Replace the oil level and filter plug.

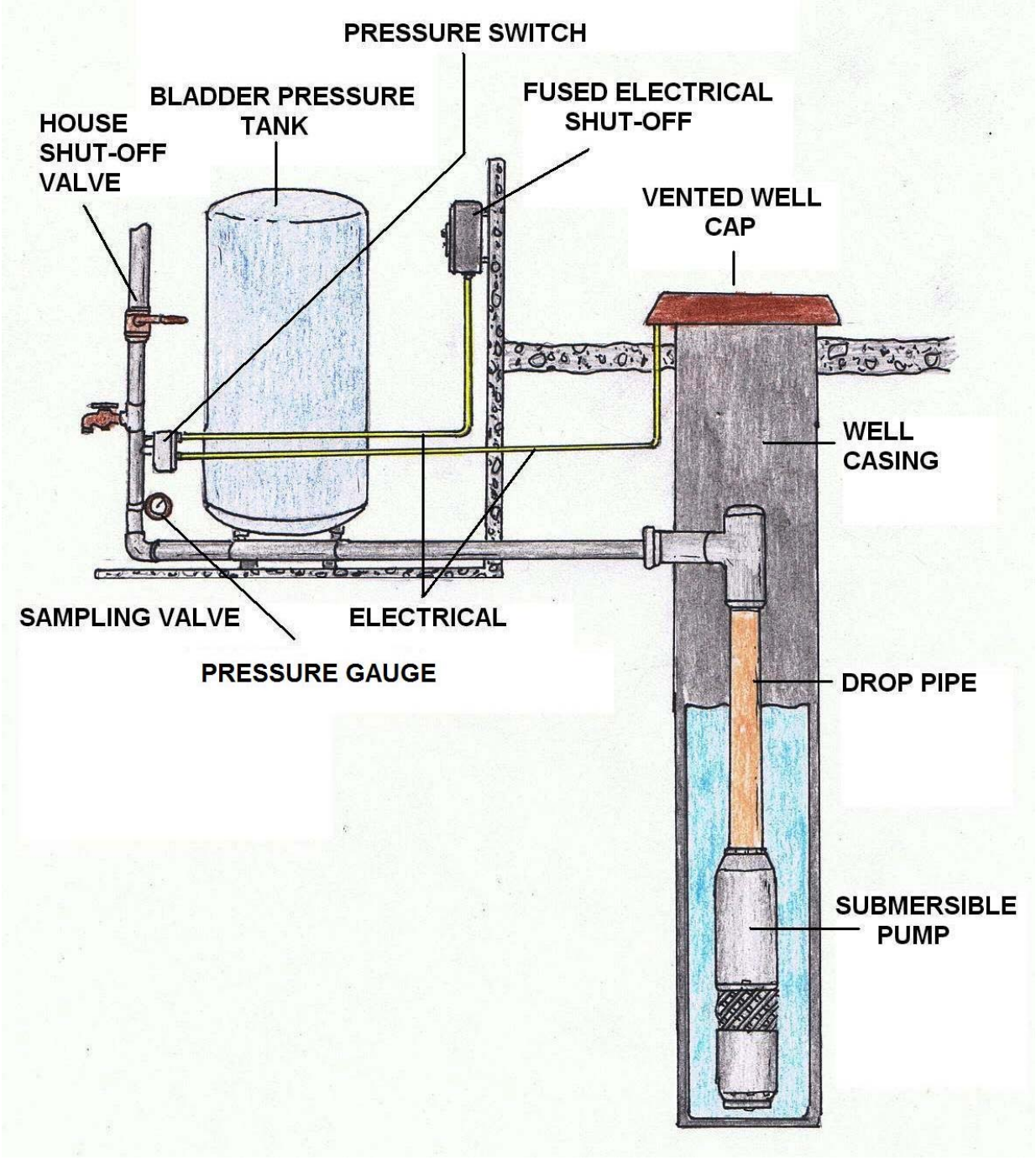
Never mix oils, since the additives of different oils when combined can cause breakdown of the oil.



Finger is shown pointing to a Lantern Ring. This old school method of sealing a pump is still out there. Notice the packing on both sides of the ring. The packing joints need to be staggered and the purpose of this device is to allow air to the Stuffing Box.

Pump Summary

Pumps are used throughout society for a variety of purposes. Early applications include the use of the windmill or watermill to pump water. Today, the pump is used for irrigation, water supply, gasoline supply, air conditioning systems, refrigeration (usually called a compressor), chemical movement, sewage movement, flood control, marine services, etc. Because of the wide variety of applications, pumps have a plethora of shapes and sizes: from very large to very small, from handling gas to handling liquid, from high pressure to low pressure, and from high volume to low volume.



Pump Safety Regulations

It is a necessity that your safety department establishes a safety program based upon a thorough analysis of industrial hazards. Before installing and operating or performing maintenance on the pump and associated components described in this manual, it is important to ensure that it covers the hazards arising from high speed rotating machinery. It is also important that due consideration be given to those hazards which arise from the presence of electrical power, hot oil, high pressure and temperature liquids, toxic liquids and gases, and flammable liquids and gases. Proper installation and care of protective guards, shut-down devices and over pressure protection equipment must also be considered an essential part of any safety program.

Also essential are special precautionary measures to prevent the possibility of applying power to the equipment at any time when maintenance work is in progress. The prevention of rotation due to reverse flow should not be overlooked. In general, all personnel should be guided by all the basic rules of safety associated with the equipment and the process. It should be understood that the information contained in this manual does not relieve operating and maintenance personnel of the responsibility of exercising good judgment in operation and care of the pump and its components.

In the following safety procedures you will encounter the words DANGER, WARNING, CAUTION, and NOTICE. These are intended to emphasize certain areas in the interest of personal safety and satisfactory pump operation and maintenance. The definitions of these words are as follows:

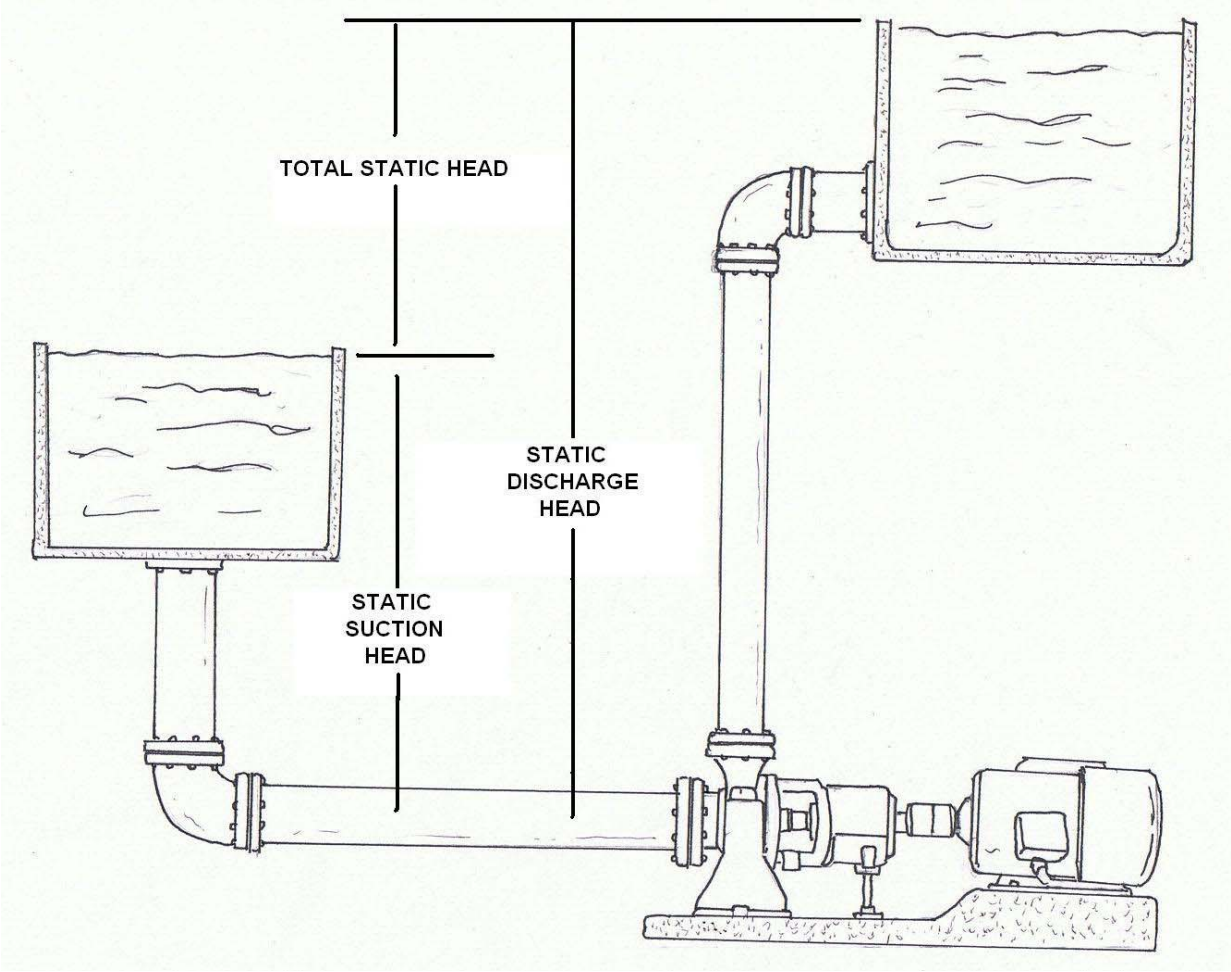
“DANGER” Danger is used to indicate the presence of a hazard which will cause severe personal injury, death, or substantial property damage if the warning is ignored.

“WARNING” Warning is used to indicate the presence of a hazard which can cause severe
personal injury, death, or substantial property damage if the warning is ignored.

“CAUTION” Caution is used to indicate the presence of a hazard which will or can cause
minor personal injury, death, or substantial property damage if the warning is ignored.

Understanding Your Pumping System Requirements

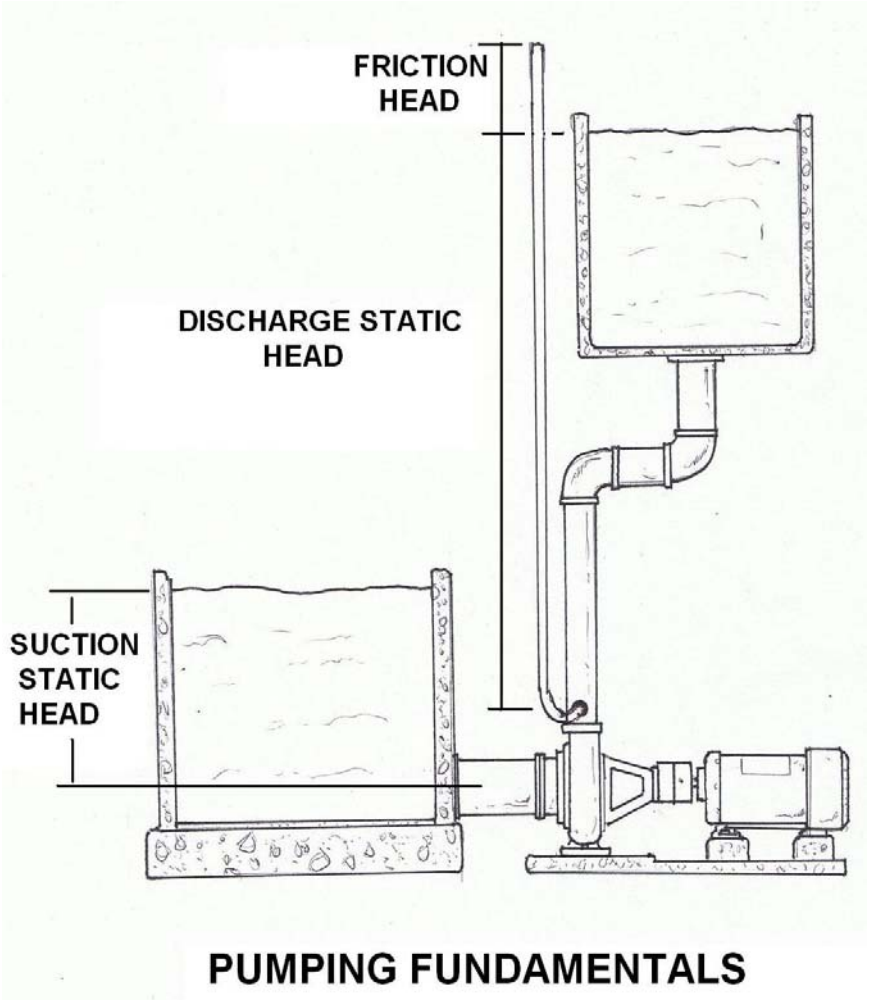
Pumps transfer liquids from one point to another by converting mechanical energy from a rotating impeller into pressure energy (head). The pressure applied to the liquid forces the fluid to flow at the required rate and to overcome friction (or head) losses in piping, valves, fittings, and process equipment. The pumping system designer must consider fluid properties, determine end use requirements, and understand environmental conditions. Pumping applications include constant or variable flow rate requirements, serving single or networked loads, and consisting of open loops (non--return or liquid delivery) or closed loops (return systems).



End Use Requirements—System Flow Rate and Head

The design pump capacity, or desired pump discharge in gallons per minute (gpm) is needed to accurately size the piping system, determine friction head losses, construct a system curve, and select a pump and drive motor. Process requirements may be met by providing a constant flow rate (with on/off control and storage used to satisfy variable flow rate requirements), or by using a throttling valve or variable speed drive to supply continuously variable flow rates.

The total system head has three components: static head, elevation (potential energy), and velocity (or dynamic) head. Static head is the pressure of the fluid in the system, and is the quantity measured by conventional pressure gauges. The height of the fluid level can have a substantial impact on system head. The dynamic head is the pressure required by the system to overcome head losses caused by flow rate resistance in pipes, valves, fittings, and mechanical equipment. Dynamic head losses are approximately proportional to the square of the fluid flow velocity, or flow rate. If the flow rate doubles, dynamic losses increase fourfold.



For many pumping systems, total system head requirements vary. For example, in wet well or reservoir applications, suction and static lift requirements may vary as the water surface elevations fluctuate. For return systems such as HVAC circulating water pumps, the values for the static and elevation heads equal zero. You also need to be aware of a pump's net positive suction head requirements. Centrifugal pumps require a certain amount of fluid pressure at the inlet to avoid cavitation. A rule of thumb is to ensure that the suction head available exceeds that required by the pump by at least 25% over the range of expected flow rates.

Fluid Properties

The properties of the fluids being pumped can significantly affect the choice of pump. Key considerations include:

• **Acidity/alkalinity (pH) and chemical composition.** Corrosive and acidic fluids can degrade pumps, and should be considered when selecting pump materials.
• **Operating temperature.** Pump materials and expansion, mechanical seal components, and packing materials need to be considered with pumped fluids that are hotter than 200°F.
• **Solids concentrations/particle sizes.** When pumping abrasive liquids such as industrial slurries, selecting a pump that will not clog or fail prematurely depends on particle size, hardness, and the volumetric percentage of solids.

• **Specific gravity.** The fluid specific gravity is the ratio of the fluid density to that of water under specified conditions. Specific gravity affects the energy required to lift and move the fluid, and must be considered when determining pump power requirements.
• **Vapor pressure.** A fluid's vapor pressure is the force per unit area that a fluid exerts in an effort to change phase from a liquid to a vapor, and depends on the fluid's chemical and physical properties. Proper consideration of the fluid's vapor pressure will help to minimize the risk of cavitation.
• **Viscosity.** The viscosity of a fluid is a measure of its resistance to motion. Since kinematic viscosity normally varies directly with temperature, the pumping system designer must know the viscosity of the fluid at the lowest anticipated pumping temperature. High viscosity fluids result in reduced centrifugal pump performance and increased power requirements. It is particularly important to consider pump suction-side line losses when pumping viscous fluids.
Reference Centrifugal/Vertical NPSH Margin (ANSI/HI 9.6.1-1998), www.pumps.org, Hydraulic Institute, 1998.

Environmental Considerations
Important environmental considerations include ambient temperature and humidity, elevation above sea level, and whether the pump is to be installed indoors or outdoors.

Software Tools
Most pump manufacturers have developed software or Web-based tools to assist in the pump selection process. Pump purchasers enter their fluid properties and system requirements to obtain a listing of suitable pumps. Software tools that allow you to evaluate and compare operating costs are available from private vendors.

Pumps as Public Water Supplies
One sort of pump once common worldwide was a hand-powered water pump, or 'pitcher pump'. It would be installed over a community water well that was used by people in the days before piped water supplies. Because water from pitcher pumps is drawn directly from the soil, it is more prone to contamination. If such water is not filtered and purified, consumption of it might lead to gastrointestinal or other water-borne diseases. A notorious case is the 1854 Broad Street cholera outbreak. At the time it was not known how cholera was transmitted, but physician John Snow suspected contaminated water and had the handle of the public pump he suspected removed; the outbreak then subsided.

Modern hand-operated community pumps are considered the most sustainable low-cost option for safe water supply in resource-poor settings, often in rural areas in developing countries. A hand pump opens access to deeper groundwater that is often not polluted and also improves the safety of a well by protecting the water source from contaminated buckets.

Pumps such as the Afridev pump are designed to be cheap to build and install, and easy to maintain with simple parts. However, scarcity of spare parts for these types of pumps in some regions of Africa has diminished their utility for these areas.

Pump Types come in Two Main Categories

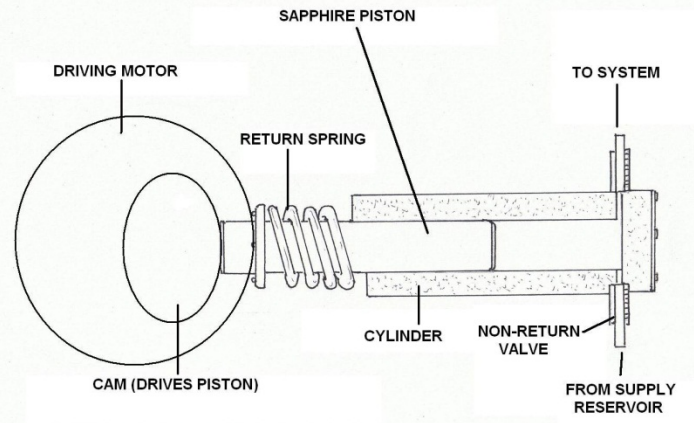
Centrifugal Pumps and Positive Displacement Pumps as classified according to the method of how the energy is imparted to the fluid – Kinetic Energy or Positive Displacement and again each of these categories having many pump types.

Centrifugal Pump
Types the Kinetic Energy type which imparts velocity energy to the pumped medium which is converted to pressure energy when discharging the pump casing and can be grouped according to several criteria, further to that a specific pump can belong to different groups.

These groups can be based upon:
The impeller suction
The number of impellers
The type of volute
International industry standards
Shaft orientation
Split case orientation
Driver pump types

Positive Displacement Pump
Types impart energy by mechanical displacement, these are of a lower flow range and are pulsating. PD pumps divided into two classes – reciprocating and rotary. Typical 'PD' pump types are:

Rotary Pump Types:
Rotary Gear Pumps
Peripheral Pumps
Screw Pumps
Gear Pumps
Rotary Lobe Pumps
Reciprocating Pump Types
Plunger Pumps
Diaphragm Pumps



PLUNGER PUMP
(single-piston reciprocating pump)

Plunger Pumps
Plunger pumps have a cylinder with a reciprocating plunger. The suction and discharge valves are mounted in the head of cylinder. The suction stroke pulls the plunger back, suction valve opens and fluid is sucked into the cylinder. The discharge stroke pushes the plunger forward closing suction valve and pushing fluid out of the discharge valve.

Diaphragm Pumps
Diaphragm pump types simply put use the plunger to pressurize either air or hydraulic fluid on one side which flexes the diaphragm which increases and decreases the volumetric area in the pumping chamber, non-return check valves ensure no back flow of the fluid.

Pump Specifications

Pumps are commonly rated by horsepower, flow rate, outlet pressure in meters (or feet) of head, inlet suction in suction feet (or meters) of head. The head can be simplified as the number of feet or meters the pump can raise or lower a column of water at atmospheric pressure. From an initial design point of view, engineers often use a quantity termed the specific speed to identify the most suitable pump type for a particular combination of flow rate and head.

Pump Construction Material
The pump material can be Stainless steel (SS 316 or SS 304), cast iron etc. It depends on the application of the pump. In the water industry and for pharma applications SS 316 is normally used, as stainless steel gives better results at high temperatures.

Pumping Power
The power imparted into a fluid will increase the energy of the fluid per unit volume. Thus the power relationship is between the conversion of the mechanical energy of the pump mechanism and the fluid elements within the pump. In general, this is governed by a series of simultaneous differential equations, known as the Navier-Stokes equations. However a more simple equation relating only the different energies in the fluid, known as Bernoulli's equation can be used.

Hence the power, P, required by the pump:

$$P = \frac{\Delta P Q}{\eta}$$

where ΔP is the change in total pressure between the inlet and outlet (in Pa), and Q, the fluid flowrate is given in m^3/s. The total pressure may have gravitational, static pressure and kinetic energy components; i.e. energy is distributed between change in the fluid's gravitational potential energy (going up or down hill), change in velocity, or change in static pressure. η is the pump efficiency, and may be given by the manufacturer's information, such as in the form of a pump curve, and is typically derived from either fluid dynamics simulation (i.e. solutions to the Navier-stokes for the particular pump geometry), or by testing. The efficiency of the pump will depend upon the pump's configuration and operating conditions (such as rotational speed, fluid density and viscosity etc.)

$$\Delta P = \frac{(v_2^2 - v_1^2)}{2} + \Delta z g + \frac{\Delta p_{\mathrm{static}}}{\rho}$$

For a typical "pumping" configuration, the work is imparted

Suction Lift Chart

The vertical distance that a pump may be placed above the water level (and be able to draw water) is determined by pump design and limits dictated by altitude. The chart below shows the absolute limits. The closer the pump is to the water level, the easier and quicker it will be to prime.

Suction Lift at Various Elevations

Altitude:	Suction Lift In Feet
Sea Level	25.0
2,000 ft.	22.0
4,000 ft.	19.5
6,000 ft.	17.3
8,000 ft.	15.5
10,000 ft.	14.3

Centrifugal pumps are particularly vulnerable especially when pumping heated solution near the vapor pressure, whereas positive displacement pumps are less affected by cavitation, as they are better able to pump two-phase flow (the mixture of gas and liquid), however, the resultant flow rate of the pump will be diminished because of the gas volumetrically displacing a disproportion of liquid. Careful design is required to pump high temperature liquids with a centrifugal pump when the liquid is near its boiling point.

The violent collapse of the cavitation bubble creates a shock wave that can literally carve material from internal pump components (usually the leading edge of the impeller) and creates noise often described as "pumping gravel".

Additionally, the inevitable increase in vibration can cause other mechanical faults in the pump and associated equipment.

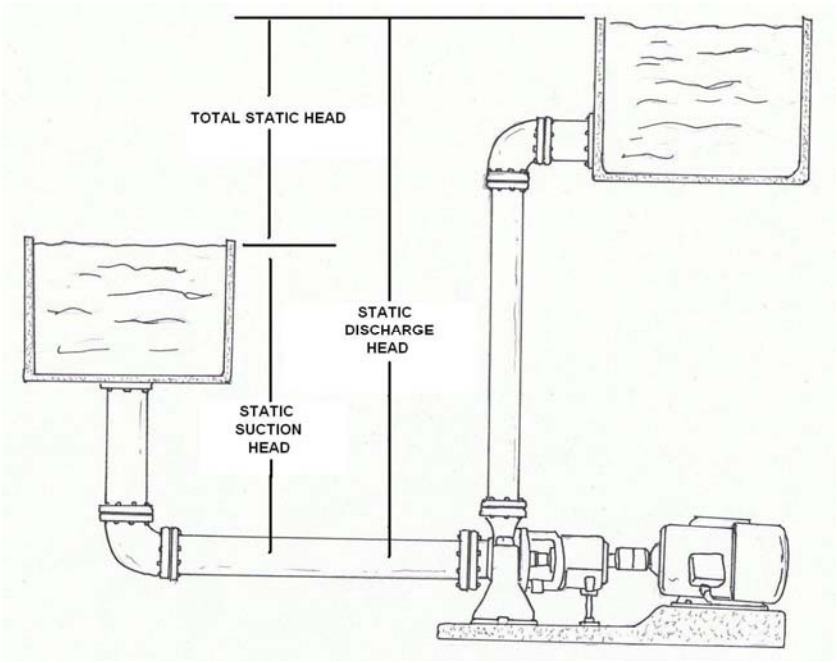
For a typical "pumping" configuration, the work is imparted on the fluid, and is thus positive. For the fluid imparting the work on the pump (i.e. a turbine), the work is negative power required to drive the pump is determined by dividing the output power by the pump efficiency. Furthermore, this definition encompasses pumps with no moving parts, such as a siphon.

When asked how a pump operates, most reply that it “sucks.” While not a false statement, it’s easy to see why so many pump operators still struggle with pump problems. Fluid flows from areas of high pressure to areas of low pressure. Pumps operate by creating low pressure at the inlet which allows the liquid to be pushed into the pump by atmospheric or head pressure (pressure due to the liquid’s surface being above the centerline of the pump). Consider placing a pump at the top of the mercury barometer above: Even with a perfect vacuum at the pump inlet, atmospheric pressure limits how high the pump can lift the liquid. With liquids lighter than mercury, this lift height can increase, but there’s still a physical limit to pump operation based on pressure external to the pump. This limit is the key consideration for Net Positive Suction Head. *Reference Centrifugal/Vertical NPSH Margin (ANSI/HI 9.6.1-1998), www.pumps.org, Hydraulic Institute, 1998.*

Pump Efficiency

Pump efficiency is defined as the ratio of the power imparted on the fluid by the pump in relation to the power supplied to drive the pump. Its value is not fixed for a given pump; efficiency is a function of the discharge and therefore also operating head. For centrifugal pumps, the efficiency tends to increase with flow rate up to a point midway through the operating range (peak efficiency) and then declines as flow rates rise further. Pump performance data such as this is usually supplied by the manufacturer before pump selection. Pump efficiencies tend to decline over time due to wear (e.g. increasing clearances as impellers reduce in size).

When a system design includes a centrifugal pump, an important issue it its design is matching the head loss-flow characteristic with the pump so that it operates at or close to the point of its maximum efficiency. Pump efficiency is an important aspect and pumps should be regularly tested. Thermodynamic pump testing is one method.



Depending on how the measurement is taken suction lift and head may also be referred to as static or dynamic. Static indicates the measurement does not take into account the friction caused by water moving through the hose or pipes. Dynamic indicates that losses due to friction are factored into the performance. The following terms are usually used when referring to lift or head.

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Dynamic Suction Head - The Static Suction Lift plus the friction in the suction line. Also referred to as a Total Suction Head.

Dynamic Discharge Head - The Static Discharge Head plus the friction in the discharge line. Also referred to as Total Discharge Head.

Total Dynamic Head - The Dynamic Suction Head plus the Dynamic Discharge Head. Also referred to as Total Head.

Net Positive Suction Head (NPSH)
NPSH can be defined as two parts:

NPSH Available (NPSHA): The absolute pressure at the suction port of the pump.

AND

NPSH Required (NPSHR): The minimum pressure required at the suction port of the pump to keep the pump from cavitating.

NPSHA is a function of your system and must be calculated, whereas NPSHR is a function of the pump and must be provided by the pump manufacturer. NPSHA MUST be greater than NPSHR for the pump system to operate without cavitating. Put another way, you must have more suction side pressure available than the pump requires.

Specific Gravity
The term specific gravity compares the density of some substance to the density of water. Since specific gravity is the ratio of those densities, the units of measure cancel themselves, and we end up with a dimensionless number that is the same for all systems of measure. Therefore, the specific gravity of water is 1— regardless of the measurement system. Specific gravity is important when sizing a centrifugal pump because it is indicative of the weight of the fluid, and its weight will have a direct effect on the amount of work performed by the pump. One of the beauties of the centrifugal pump is that the head (in feet) and flow it produces has nothing to do with the weight of the liquid. It is all about the velocity that is added by the impeller. The simplest way to prove the validity of this statement is to use the falling body equation:

$v2 = 2gh$

Where:
v = Velocity
g = The universal gravitational constant
h = height.

This equation will predict the final velocity some object will attain when falling from some height (ignoring friction of course). When rearranged, it takes the form of $h = v2/2g$ and predicts the maximum height an object can attain based on its initial velocity. The final velocity attained by a falling object is actually the same as the initial velocity required for it to rise to the same height from which it fell.

When this equation is applied to a centrifugal pump, h becomes the maximum theoretical head that it can produce. As the equation illustrates, that head depends upon the exit velocity of the liquid from the impeller vanes and the effect of gravity; it has absolutely nothing to do with the weight of the liquid.

The weight of the liquid does affect the amount of work done by a pump and, therefore, the HP required. A good way to understand the impact of liquid weight is to convert flow in GPM and head in feet into units of work. The equation below performs this conversion.

$(gpm \times 8.34\ lb/gal \times h) = w$

Here the flow is multiplied by the weight of a gallon of water and then multiplied by the head in feet. The result is the work performed in ft-lb/minute. The equation shows us that the amount of work done by a centrifugal pump is directly proportional to the weight of the pumped liquid. If you divide w by 33,000, the result is the HP required at that particular point of flow and head. The downward sloping curve in the upper portion of the graph is the H/Q curve and the red, blue and green curves are the horsepower curves for three different liquids. The scale of the Y axis is both head and horsepower. The blue curve shows the HP required for water (SG=1). The red and green curves show the HP required to pump sugar syrup (SG=1.29) and gasoline (SG=0.71). If you analyze the three HP curves at each flow point, you will see that the increase or decrease is directly proportional to the SG of that particular liquid.

As long as the viscosity of a liquid is similar to that of water, its specific gravity will have no effect on pump performance. It will, however, directly affect the input power required to pump that particular liquid. The equation below can be used to compute the horsepower required to pump liquids of varying specific gravities (where BHP is brake horsepower, Q is flow in GPM, H is head in feet, SG is specific gravity and Eff is the hydraulic efficiency of the pump). It assumes a viscosity similar to that of water.

BHP = (Q x H x SG) / (3960 x Eff)

SG can also have an effect on the onset of cavitation in a particular pump. Heavier liquids cause a proportional increase in a pump's suction energy and those with a high suction energy level are more likely to experience cavitation damage. Next month we will review the effect of viscosity on centrifugal pump performance.

Pump Testing

To minimize energy use, and to ensure that pumps are correctly matched to the duty expected pumps, and pumping stations should be regularly tested. In water supply applications, which are usually fitted with centrifugal pumps, individual large pumps should be 70 - 80% efficient. They should be individually tested to ensure they are in the appropriate range, and replaced or prepared as appropriate. Pumping stations should also be tested collectively, because where pumps can run in combination to meet a given demand, it is often possible for very inefficient combination of pumps to occur. For example: it is perfectly possible to have a large and a small pump operating in parallel, with the smaller pump not delivering any water, but merely consuming energy. Pumps are readily tested by fitting a flow meter, measuring the pressure difference between inlet and outlet, and measuring the power consumed. Another method is thermodynamic pump testing where only the temperature rise and power consumed need be measured.

Depending on how the measurement is taken suction lift and head may also be referred to as static or dynamic. Static indicates the measurement does not take into account the friction caused by water moving through the hose or pipes. Dynamic indicates that losses due to friction are factored into the performance. The following terms are usually used when referring to lift or head.

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Total Dynamic Head - The Dynamic Suction Head plus the Dynamic Discharge Head. Also referred to as Total Head.

Positive Displacement Pumps

A positive displacement pump causes a fluid to move by trapping a fixed amount of it and then forcing (displacing) that trapped volume into the discharge pipe. Some positive displacement pumps work using an expanding cavity on the suction side and a decreasing cavity on the discharge side. Liquid flows into the pump as the cavity on the suction side expands and the liquid flows out of the discharge as the cavity collapses. The volume is constant given each cycle of operation.

Positive Displacement Pump Behavior and Safety
Positive displacement pumps, unlike centrifugal or roto-dynamic pumps, will in theory produce the same flow at a given speed (RPM) no matter what the discharge pressure. Thus, positive displacement pumps are "constant flow machines". However due to a slight increase in internal leakage as the pressure increases, a truly constant flow rate cannot be achieved.

A positive displacement pump must not be operated against a closed valve on the discharge side of the pump, because it has no shut-off head like centrifugal pumps. A positive displacement pump operating against a closed discharge valve will continue to produce flow and the pressure in the discharge line will increase, until the line bursts or the pump is severely damaged, or both.

A relief or safety valve on the discharge side of the positive displacement pump is therefore necessary. The relief valve can be internal or external. The pump manufacturer normally has the option to supply internal relief or safety valves. The internal valve should in general only be used as a safety precaution, an external relief valve installed in the discharge line with a return line back to the suction line or supply tank is recommended.

Priming a Pump
Liquid and slurry pumps can lose prime and this will require the pump to be primed by adding liquid to the pump and inlet pipes to get the pump started. Loss of "prime" is usually due to ingestion of air into the pump. The clearances and displacement ratios in pumps used for liquids and other more viscous fluids cannot displace the air due to its lower density.

Positive Displacement Types
A positive displacement pump causes a liquid or gas to move by trapping a fixed amount of fluid or gas and then forcing (displacing) that trapped volume into the discharge pipe. Positive displacement pumps can be further classified as either rotary-type (for example the rotary vane) or lobe pumps similar to oil pumps used in car engines. Moreover, these pumps give a non-pulsating output or displacement unlike the reciprocating pumps and hence are called positive displacement pumps.

The positive displacement pump operates by alternating of filling a cavity and then displacing a given volume of liquid. The positive displacement pump delivers a constant volume of liquid for each cycle against varying discharge pressure or head.

The positive displacement pump can be classified as:

- ✓ Reciprocating pumps - piston, plunger and diaphragm
- ✓ Power pumps
- ✓ Steam pumps
- ✓ Rotary pumps - gear, lobe, screw, vane, regenerative (peripheral) and progressive cavity

A positive displacement pump can be further classified according to the mechanism used to move the fluid:

- ✓ Rotary-type positive displacement: internal gear, screw, shuttle block, flexible vane or sliding vane, circumferential piston, flexible impeller, helical twisted roots (e.g. the Wendelkolben pump) or liquid ring vacuum pumps.
- ✓ Reciprocating-type positive displacement: piston or diaphragm pumps.
- ✓ Linear-type positive displacement: rope pumps and chain pumps.

Rotary Positive Displacement Pumps

Positive displacement rotary pumps move fluid using a rotating mechanism that creates a vacuum that captures and draws in the liquid.

Advantages: Rotary pumps are very efficient because they naturally remove air from the lines, eliminating the need to bleed the air from the lines manually.

Drawbacks: Because of the nature of the pump, the clearance between the rotating pump and the outer edge must be very close, requiring that it rotate at a slow, steady speed. If rotary pumps are operated at high speeds, the fluids will cause erosion, eventually developing enlarged clearances through which liquid can pass, reducing the efficiency of the pump.

Rotary positive displacement pumps can be grouped into three main types:

- ✓ Gear pumps - a simple type of rotary pump where the liquid is pushed between two gears.
- ✓ Screw pumps - the shape of the internals of this pump usually two screws turning against each other pump the liquid.
- ✓ Rotary vane pumps - similar to scroll compressors, consisting of a cylindrical rotor encased in a similarly shaped housing. As the rotor orbits, the vanes trap fluid between the rotor and the casing, drawing the fluid through the pump.

Reciprocating Positive Displacement Pumps

Hand-operated, reciprocating, positive displacement, and Slovakia (walking beam pump).

Reciprocating pumps are those which cause the fluid to move using one or more oscillating pistons, plungers or membranes (diaphragms), and restrict motion of the fluid to the one desired direction by valves.

Pumps in this category range from "simplex", with one cylinder, to in some cases "quad" (four) cylinders or more. Many reciprocating-type pumps are "duplex" (two) or "triplex" (three) cylinder. They can be either "single-acting" with suction during one direction of piston motion and discharge on the other, or "double-acting" with suction and discharge in both directions. The pumps can be powered manually, by air or steam, or by a belt driven by an engine. This type of pump was used extensively in the early days of steam propulsion (19th century) as boiler feed water pumps. Reciprocating pumps are now typically used for pumping highly viscous fluids including concrete and heavy oils, and special applications demanding low flow rates against high resistance. Reciprocating hand pumps were widely used for pumping water from wells; the common bicycle pump and foot pumps for inflation use reciprocating action.
These positive displacement pumps have an expanding cavity on the suction side and a decreasing cavity on the discharge side. Liquid flows into the pumps as the cavity on the suction side expands and the liquid flows out of the discharge as the cavity collapses. The volume is constant given each cycle of operation.

Typical reciprocating pumps are:

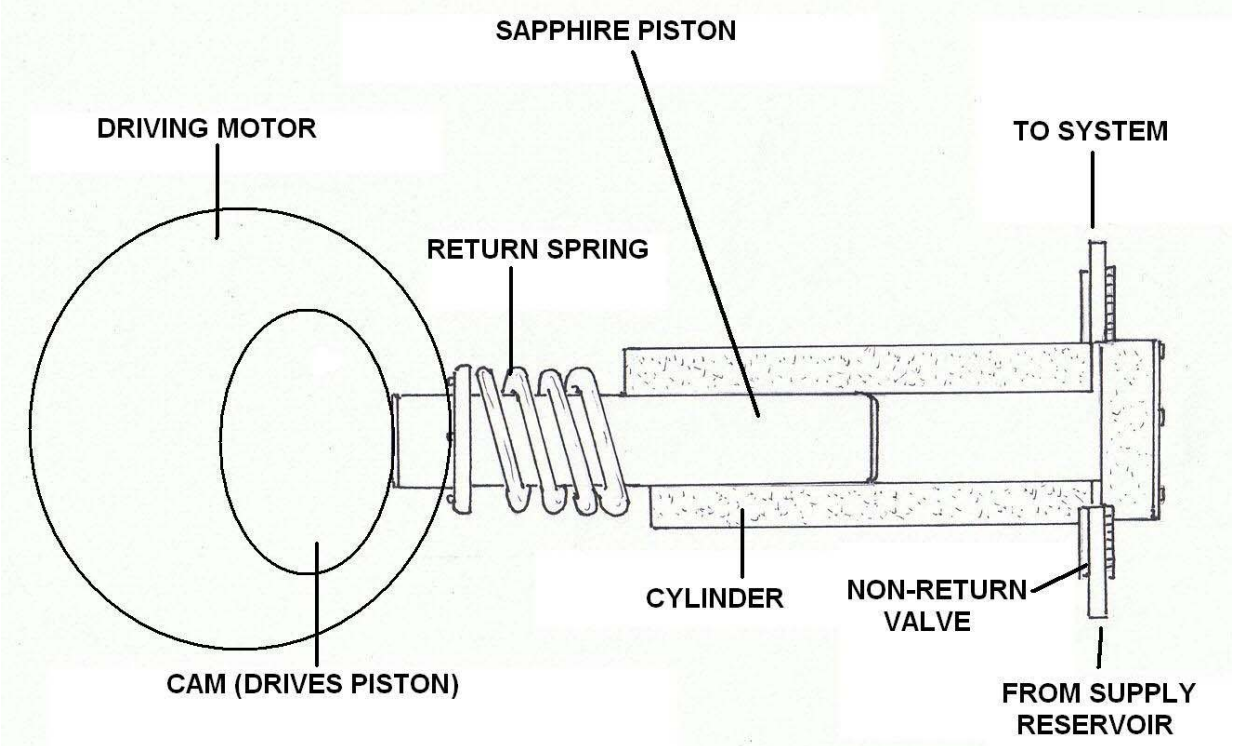
- ✓ Plunger pumps - a reciprocating plunger pushes the fluid through one or two open valves, closed by suction on the way back.
- ✓ Diaphragm pumps - similar to plunger pumps, where the plunger pressurizes hydraulic oil which is used to flex a diaphragm in the pumping cylinder. Diaphragm valves are used to pump hazardous and toxic fluids.
- ✓ Piston displacement pumps - usually simple devices for pumping small amounts of liquid or gel manually. An example is the common hand soap pump.
- ✓ Radial piston pump

Various Positive Displacement Pumps

The positive displacement principle applies in the following types of pumps:

- ✓ Rotary lobe pump
- ✓ Progressive cavity pump
- ✓ Rotary gear pump
- ✓ Piston pump
- ✓ Diaphragm pump
- ✓ Screw pump
- ✓ Gear pump
- ✓ Hydraulic pump

- ✓ Vane pump
- ✓ Regenerative (peripheral) pump
- ✓ Peristaltic pump
- ✓ Rope pump
- ✓ Flexible impeller



PLUNGER PUMP
(single-piston reciprocating pump)

Centrifugal or Roto-Dynamic Pump
The centrifugal or roto-dynamic pump produce a head and a flow by increasing the velocity of the liquid through the machine with the help of a rotating vane impeller. Centrifugal pumps include radial, axial and mixed flow units.

Centrifugal pumps can further be classified as
- ✓ end suction pumps
- ✓ in-line pumps
- ✓ double suction pumps

- ✓ vertical multistage pumps
- ✓ horizontal multistage pumps
- ✓ submersible pumps
- ✓ self-priming pumps
- ✓ axial-flow pumps
- ✓ regenerative pumps

The fact of the matter is that there are three types of problems mostly encountered with centrifugal pumps:

- ✓ design errors
- ✓ poor operation
- ✓ poor maintenance practices

Working Mechanism of a Centrifugal Pump

A centrifugal pump is one of the simplest pieces of equipment in any process plant. Its purpose is to convert energy of a prime mover (an electric motor or turbine) first into velocity or kinetic energy and then into pressure energy of a fluid that is being pumped. The energy changes occur by virtue of two main parts of the pump, the impeller and the volute or diffuser. The impeller is the rotating part that converts driver energy into the kinetic energy. The volute or diffuser is the stationary part that converts the kinetic energy into pressure energy.

Note: All of the forms of energy involved in a liquid flow system are expressed in terms of feet of liquid i.e. head.

Generation of Centrifugal Force

The process liquid enters the suction nozzle and then into eye (center) of a revolving device known as an impeller. When the impeller rotates, it spins the liquid sitting in the cavities between the vanes outward and provides centrifugal acceleration. As liquid leaves the eye of the impeller a low-pressure area is created causing more liquid to flow toward the inlet. Because the impeller blades are curved, the fluid is pushed in a tangential and radial direction by the centrifugal force. This force acting inside the pump is the same one that keeps water inside a bucket that is rotating at the end of a string.

Selecting between Centrifugal or Positive Displacement Pumps

Selecting between a Centrifugal Pump or a Positive Displacement Pump is not always straight forward.

Flow Rate and Pressure Head

The two types of pumps behave very differently regarding pressure head and flow rate: The Centrifugal Pump has varying flow depending on the system pressure or head. The Positive Displacement Pump has more or less a constant flow regardless of the system pressure or head. Positive Displacement pumps generally gives more pressure than

Centrifugal Pump's. Depending on how the measurement is taken suction lift and head may also be referred to as static or dynamic. Static indicates the measurement does not take into account the friction caused by water moving through the hose or pipes. Dynamic indicates that losses due to friction are factored into the performance. The following terms are usually used when referring to lift or head.

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Total Dynamic Head - The Dynamic Suction Head plus the Dynamic Discharge Head. Also referred to as Total Head.

Capacity and Viscosity
Another major difference between the pump types is the effect of viscosity on the capacity:

- ✓ In the Centrifugal Pump the flow is reduced when the viscosity is increased.
- ✓ In the Positive Displacement Pump the flow is increased when viscosity is increased

Liquids with high viscosity fills the clearances of a Positive Displacement Pump causing a higher volumetric efficiency and a Positive Displacement Pump is better suited for high viscosity applications. A Centrifugal Pump becomes very inefficient at even modest viscosity.

Mechanical Efficiency
The pumps behaves different considering mechanical efficiency as well.

- ✓ Changing the system pressure or head has little or no effect on the flow rate in the Positive Displacement Pump.
- ✓ Changing the system pressure or head has a dramatic effect on the flow rate in the Centrifugal Pump.

Net Positive Suction Head - NPSH
Another consideration is the Net Positive Suction Head NPSH.

- ✓ In a Centrifugal Pump, NPSH varies as a function of flow determined by pressure.

- ✓ In a Positive Displacement Pump, NPSH varies as a function of flow determined by speed. Reducing the speed of the Positive Displacement Pump, reduces the NPSH.

Darcy-Weisbach Formula
Flow of fluid through a pipe
The flow of liquid through a pipe is resisted by viscous shear stresses within the liquid and the turbulence that occurs along the internal walls of the pipe, created by the roughness of the pipe material. This resistance is usually known as pipe friction and is measured is feet or meters head of the fluid, thus the term head loss is also used to express the resistance to flow.

Many factors affect the head loss in pipes, the viscosity of the fluid being handled, the size of the pipes, the roughness of the internal surface of the pipes, the changes in elevations within the system and the length of travel of the fluid. The resistance through various valves and fittings will also contribute to the overall head loss. A method to model the resistances for valves and fittings is described elsewhere. In a well-designed system the resistance through valves and fittings will be of minor significance to the overall head loss, many designers choose to ignore the head loss for valves and fittings at least in the initial stages of a design.

Much research has been carried out over many years and various formulas to calculate head loss have been developed based on experimental data. Among these is the Chézy formula which dealt with water flow in open channels. Using the concept of 'wetted perimeter' and the internal diameter of a pipe the Chézy formula could be adapted to estimate the head loss in a pipe, although the constant 'C' had to be determined experimentally.

The Darcy-Weisbach equation
Weisbach first proposed the equation we now know as the Darcy-Weisbach formula or Darcy-Weisbach equation:

$hf = f (L/D) \times (v2/2g)$

where:
hf = head loss (m)
f = friction factor
L = length of pipe work (m)
d = inner diameter of pipe work (m)
v = velocity of fluid (m/s)
g = acceleration due to gravity (m/s^2)
or:
hf = head loss (ft)
f = friction factor
L = length of pipe work (ft)
d = inner diameter of pipe work (ft)
v = velocity of fluid (ft/s)
g = acceleration due to gravity (ft/s^2)

The Moody Chart
In 1944 LF Moody plotted the data from the Colebrook equation and this chart which is now known as 'The Moody Chart' or sometimes the Friction Factor Chart, enables a user to plot the Reynolds number and the Relative Roughness of the pipe and to establish a reasonably accurate value of the friction factor for turbulent flow conditions.

The Moody Chart encouraged the use of the Darcy-Weisbach friction factor and this quickly became the method of choice for hydraulic engineers. Many forms of head loss calculator were developed to assist with the calculations, amongst these a round slide rule offered calculations for flow in pipes on one side and flow in open channels on the reverse side.

The development of the personnel computer from the 1980's onwards reduced the time needed to perform the friction factor and head loss calculations, which in turn has widened the use of the Darcy-Weisbach formula to the point that all other formula are now largely unused.

This dimensionless chart is used to work out pressure drop, ΔP(Pa) (or head loss, h_f (m)) and flow rate through pipes. Head loss can be calculated using the Darcy–Weisbach equation:

$$h_f = f \frac{l}{d} \frac{V^2}{2g};$$

not to be confused with the Fanning equation and the Fanning friction factor:

$$h_f = 4f \frac{l}{d} \frac{V^2}{2g},$$

which uses a friction-factor equal to one fourth the Darcy-Weisbach friction factor. Pressure drop can then be evaluated as:

$\Delta P = \rho g h_f$ or directly from $\Delta P = f \frac{\rho V^2}{2} \frac{l}{d},$

where ρ is the density of the fluid, V is the average velocity in the pipe, f is the friction factor from the Moody chart, l is the length of the pipe and d is the pipe diameter.
The basic chart plots Darcy–Weisbach friction factor against Reynolds number for a variety of relative roughnesses and flow regimes. The relative roughness being the ratio of the mean height of roughness of the pipe to the pipe diameter or $\frac{\epsilon}{d}$.
The Moody chart can be divided into two regimes of flow: laminar and turbulent. For the laminar flow regime, the Darcy–Weisbach friction factor was determined analytically by

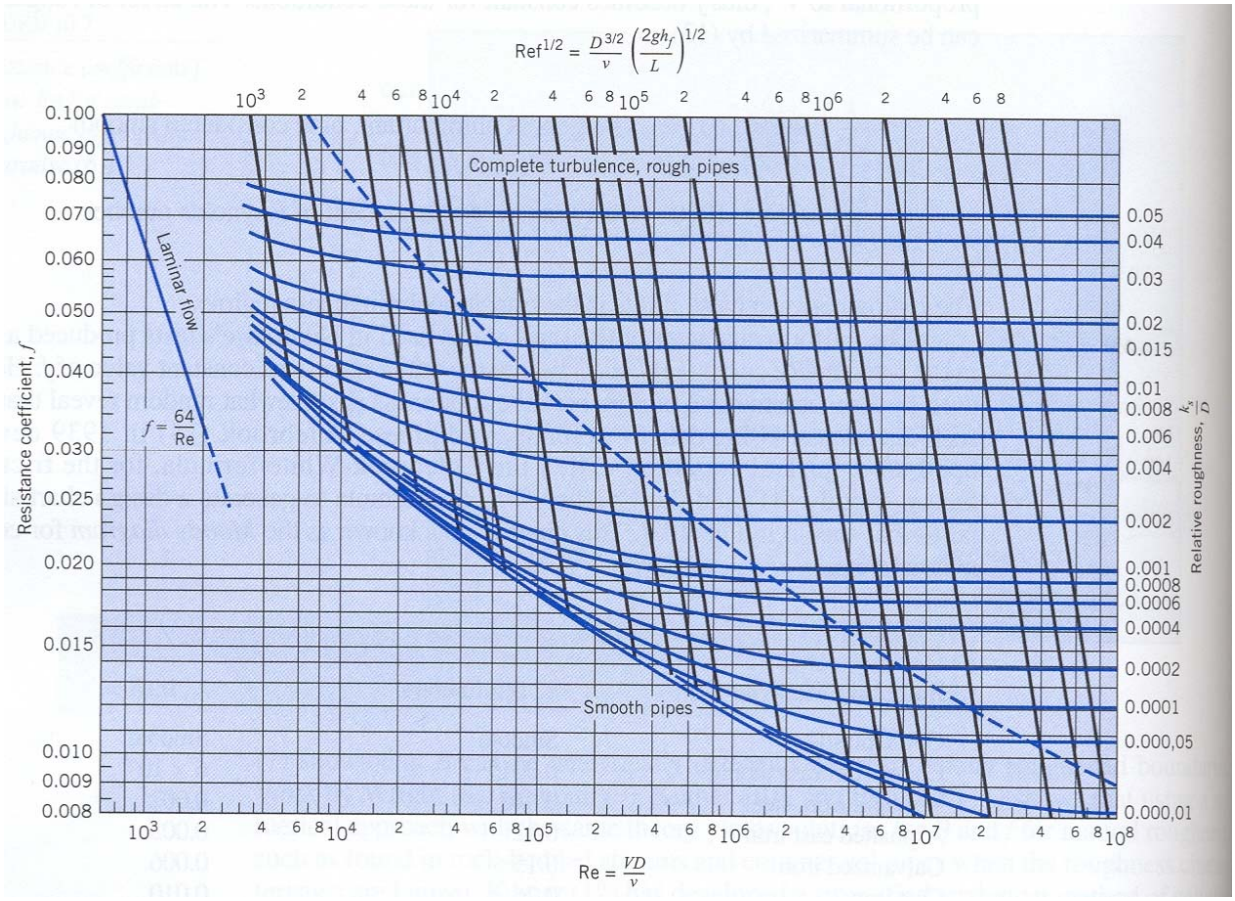
$$\frac{64}{Re}$$

Poiseuille and is used. In this regime roughness has no discernible effect. For the turbulent flow regime, the relationship between the friction factor and the Reynolds number is more complex and is governed by the Colebrook equation which is implicit in f:

$$\frac{1}{\sqrt{f}} = -2.0 \log_{10}\left(\frac{\frac{\epsilon}{d}}{3.7} + \frac{2.51}{Re\sqrt{f}}\right), \text{turbulent flow.}$$

In 1944, Lewis Ferry Moody plotted the Darcy–Weisbach friction factor into what is now known as the Moody chart.

The Fanning friction factor is 1/4 the Darcy–Weisbach one and the equation for pressure drop has a compensating factor of four.



Peristaltic Pumps

A peristaltic pump is a type of positive displacement pump used for pumping a variety of fluids. The fluid is contained within a flexible tube fitted inside a circular pump casing (though linear peristaltic pumps have been made). A rotor with a number of "rollers", "shoes" or "wipers" attached to the external circumference compresses the flexible tube. As the rotor turns, the part of the tube under compression closes (or "occludes") thus forcing the fluid to be pumped to move through the tube. Additionally, as the tube opens to its natural state after the passing of the cam ("restitution") fluid flow is induced to the pump. This process is called peristalsis and is used in many biological systems such as the gastrointestinal tract.

Priming a Pump
Liquid and slurry pumps can lose prime and this will require the pump to be primed by adding liquid to the pump and inlet pipes to get the pump started. Loss of "prime" is usually due to ingestion of air into the pump. The clearances and displacement ratios in pumps used for liquids and other more viscous fluids cannot displace the air due to its lower density.

Plunger Pumps
Plunger pumps are reciprocating positive displacement pumps. They consist of a cylinder with a reciprocating plunger in them. The suction and discharge valves are mounted in the head of the cylinder. In the suction stroke the plunger retracts and the suction valves open causing suction of fluid into the cylinder. In the forward stroke the plunger pushes the liquid out of the discharge valve.

Efficiency and Common Problems
With only one cylinder in plunger pumps, the fluid flow varies between maximum flow when the plunger moves through the middle positions and zero flow when the plunger is at the end positions. A lot of energy is wasted when the fluid is accelerated in the piping system. Vibration and "water hammer" may be a serious problem. In general the problems are compensated for by using two or more cylinders not working in phase with each other.

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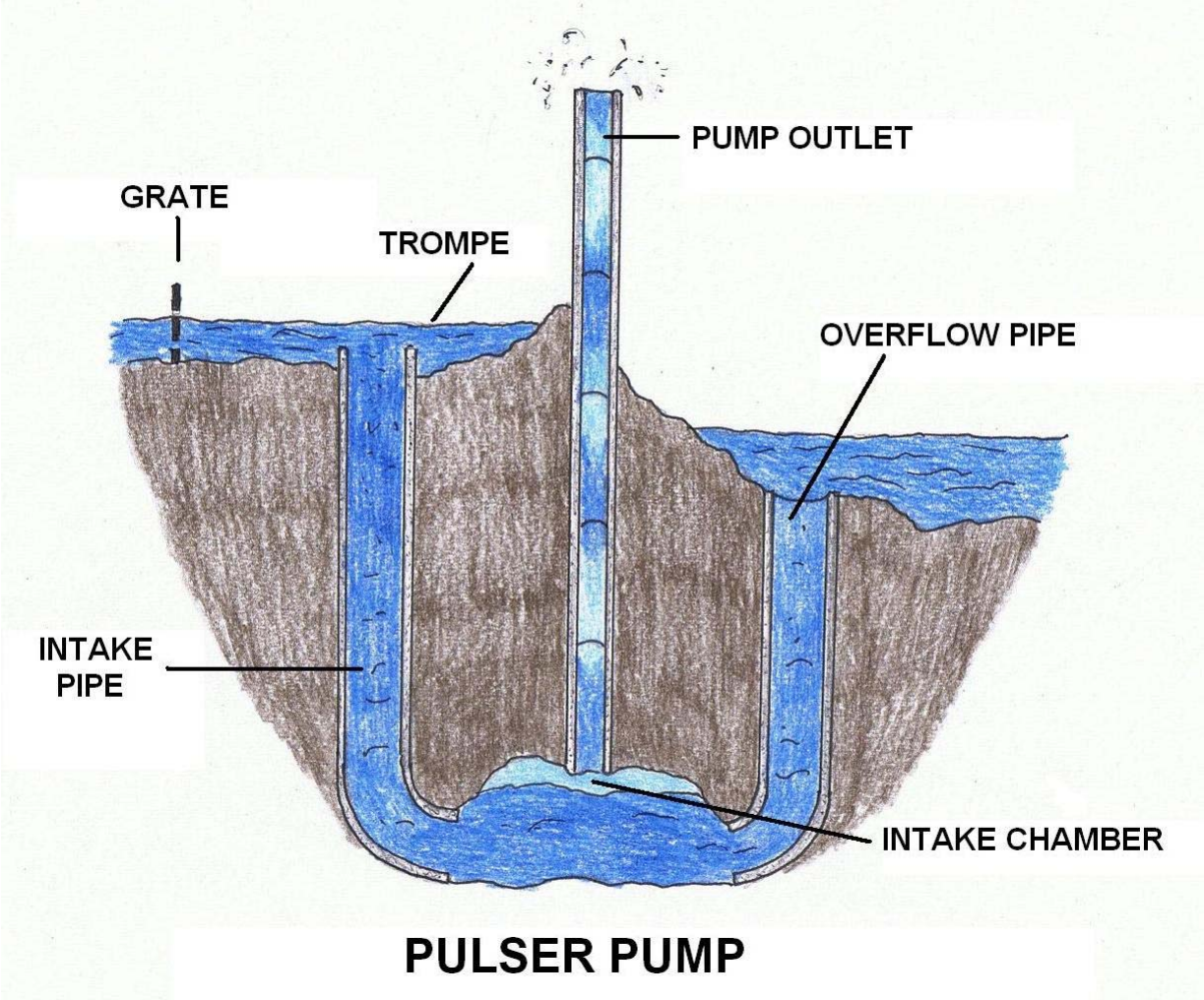
Compressed-Air-Powered Double-Diaphragm Pumps
One modern application of positive displacement diaphragm pumps is compressed-air-powered double-diaphragm pumps. Run on compressed air these pumps are intrinsically safe by design, although all manufacturers offer ATEX certified models to comply with industry regulation. Commonly seen in all areas of industry from shipping to processing, Wilden Pumps, Graco, SandPiper or ARO are generally the larger of the brands. They are relatively inexpensive and can be used for almost any duty from pumping water out of bunds, to pumping hydrochloric acid from secure storage (dependent on how the pump is manufactured – elastomers / body construction). Lift is normally limited to roughly 6m although heads can reach almost 200 Psi.

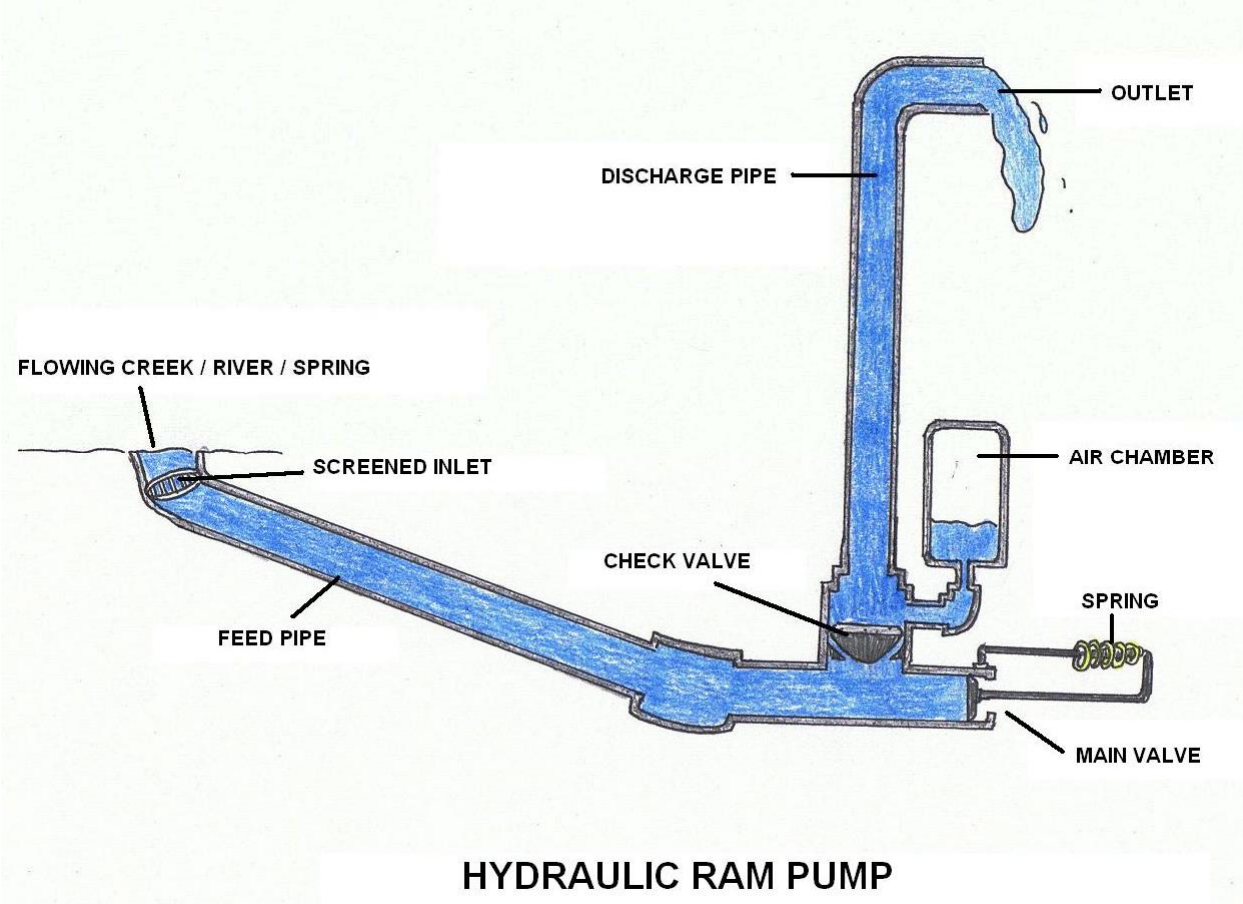
Impulse Pumps

Impulse pumps use pressure created by gas (usually air). In some impulse pumps the gas trapped in the liquid (usually water), is released and accumulated somewhere in the pump, creating a pressure which can push part of the liquid upwards.



Impulse pumps include:

- ✓ Hydraulic ram pumps - uses pressure built up internally from released gas in liquid flow.
- ✓ Pulser pumps - run with natural resources, by kinetic energy only.
- ✓ Airlift pumps - run on air inserted into pipe, pushing up the water, when bubbles move upward, or on pressure inside pipe pushing water up.



HYDRAULIC RAM PUMP

Hydraulic Ram Pumps

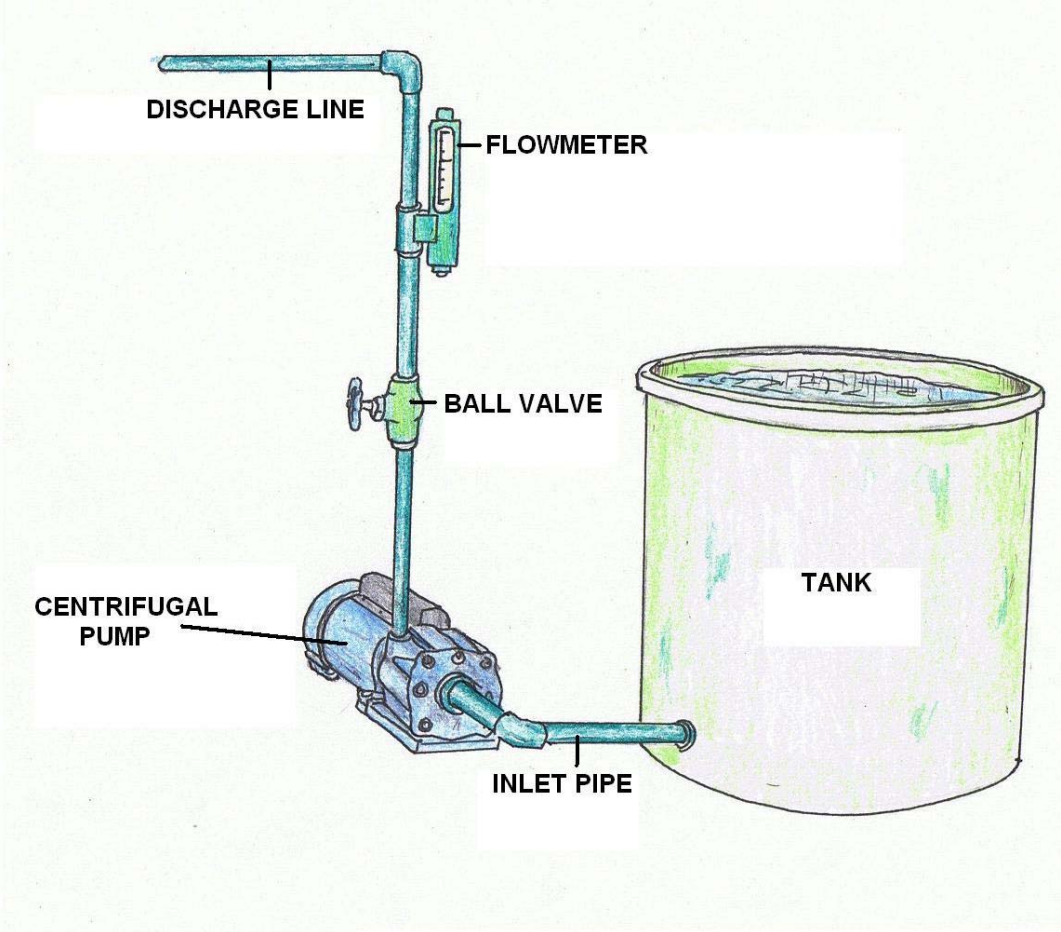
A hydraulic ram is a water pump powered by hydropower. It functions as a hydraulic transformer that takes in water at one "hydraulic head" (pressure) and flow-rate, and outputs water at a higher hydraulic-head and lower flow-rate. The device uses the water hammer effect to develop pressure that allows a portion of the input water that powers the pump to be lifted to a point higher than where the water originally started. The hydraulic ram is sometimes used in remote areas, where there is both a source of low-head hydropower, and a need for pumping water to a destination higher in elevation than the source. In this situation, the ram is often useful, since it requires no outside source of power other than the kinetic energy of flowing water.

Velocity Pumps

Rotodynamic pumps (or dynamic pumps) are a type of velocity pump in which kinetic energy is added to the fluid by increasing the flow velocity. This increase in energy is converted to a gain in potential energy (pressure) when the velocity is reduced prior to or as the flow exits the pump into the discharge pipe. This conversion of kinetic energy to pressure can be explained by the First law of thermodynamics or more specifically by Bernoulli's principle. Dynamic pumps can be further subdivided according to the means in which the velocity gain is achieved.

These types of pumps have a number of characteristics:
1. Continuous energy
2. Conversion of added energy to increase in kinetic energy (increase in velocity)
3. Conversion of increased velocity (kinetic energy) to an increase in pressure head

One practical difference between dynamic and positive displacement pumps is their ability to operate under closed valve conditions. Positive displacement pumps physically displace the fluid; hence closing a valve downstream of a positive displacement pump will result in a continual build up in pressure resulting in mechanical failure of either pipeline or pump. Dynamic pumps differ in that they can be safely operated under closed valve conditions (for short periods of time).



GRAVITY FEED SYSTEM

Gravity Pumps
Gravity pumps include the syphon and Heron's fountain – and there also important qanat or foggara systems which simply use downhill flow to take water from far-underground aquifers in high areas to consumers at lower elevations. The hydraulic ram is also sometimes referred to as a gravity pump.

Reciprocating Pumps

Typical reciprocating pumps are
• plunger pumps
• diaphragm pumps
A plunger pump consists of a cylinder with a reciprocating plunger in it. The suction and discharge valves are mounted in the head of the cylinder. In the suction stroke the plunger retracts and the suction valves open causing suction of fluid into the cylinder. In the forward stroke the plunger pushes the liquid out of the discharge valve.

With only one cylinder the fluid flow varies between maximum flow when the plunger moves through the middle positions and zero flow when the plunger is at the end positions. A lot of energy is wasted when the fluid is accelerated in the piping system. Vibration and "water hammer" may be a serious problem. In general the problems are compensated for by using two or more cylinders not working in phase with each other.

In diaphragm pumps, the plunger pressurizes hydraulic oil which is used to flex a diaphragm in the pumping cylinder. Diaphragm valves are used to pump hazardous and toxic fluids. An example of the piston displacement pump is the common hand soap pump.

Gear Pump
This uses two meshed gears rotating in a closely fitted casing. Fluid is pumped around the outer periphery by being trapped in the tooth spaces. It does not travel back on the meshed part, since the teeth mesh closely in the center. Widely used on car engine oil pumps. it is also used in various hydraulic power packs..

Progressing Cavity Pump
Widely used for pumping difficult materials such as sewage sludge contaminated with large particles, this pump consists of a helical shaped rotor, about ten times as long as its width. This can be visualized as a central core of diameter x, with typically a curved spiral wound around of thickness half x, although of course in reality it is made from one casting. This shaft fits inside a heavy duty rubber sleeve, of wall thickness typically x also. As the shaft rotates, fluid is gradually forced up the rubber sleeve. Such pumps can develop very high pressure at quite low volumes.

Diaphragm Pumps
A diaphragm pump is a positive displacement pump that uses a combination of the reciprocating action of a rubber, thermoplastic or teflon diaphragm and suitable non-return check valves to pump a fluid. Sometimes this type of pump is also called a membrane pump. Diaphragm Pumps are used extensively in many industries and can handle a very wide variety of liquids. Diaphragm Pumps are in the category of "positive displacement" pumps because their flowrates do not vary much with the discharge "head" (or pressure) the pump is working against (for a given pump speed). Diaphragm Pumps can transfer liquids with low, medium or high viscosities and also liquids with a large solids content. They can also handle many aggressive chemicals such as acids because they can be constructed with a wide variety of body materials and diaphragms.

There are three main types of diaphragm pumps:

- ✓ Those in which the diaphragm is sealed with one side in the fluid to be pumped, and the other in air or hydraulic fluid. The diaphragm is flexed, causing the volume of the pump chamber to increase and decrease. A pair of non-return check valves prevent reverse flow of the fluid.

- ✓ Those employing volumetric positive displacement where the prime mover of the diaphragm is electro-mechanical, working through a crank or geared motor drive. This method flexes the diaphragm through simple mechanical action, and one side of the diaphragm is open to air.

- ✓ Those employing one or more unsealed diaphragms with the fluid to be pumped on both sides. The diaphragm(s) again are flexed, causing the volume to change.

When the volume of a chamber of either type of pump is increased (the diaphragm moving up), the pressure decreases, and fluid is drawn into the chamber. When the chamber pressure later increases from decreased volume (the diaphragm moving down), the fluid previously drawn in is forced out. Finally, the diaphragm moving up once again draws fluid into the chamber, completing the cycle. This action is similar to that of the cylinder in an internal combustion engine. The most popular type of diaphragm pump is the Air-Operated Diaphragm Pump.

These pumps use compressed air as their power supply. They also include two chambers with a diaphragm, inlet check valve and outlet check valve in each chamber. The air supply is shifted from one chamber to another with an air spool valve that is built into the pump. This continual shifting of air from one chamber to another (to the backside of the diaphragm) forces liquid out of one chamber and into the discharge piping while the other chamber is being filled with liquid. There is some pulsation of discharge flow in Air-Operated Diaphragm Pumps. This pulsating flow can be reduced somewhat by using pulsation dampeners in the discharge piping.

Characteristics
Diaphragm Pumps:

- ✓ have good suction lift characteristics, some are low pressure pumps with low flow rates; others are capable of higher flow rates, dependent on the effective working diameter of the diaphragm and its stroke length. They can handle sludges and slurries with a relatively high amount of grit and solid content.

- ✓ suitable for discharge pressure up to 1,200 bar.

- ✓ have good dry running characteristics.

- ✓ can be used to make artificial hearts.

- ✓ are used to make air pumps for the filters on small fish tanks.

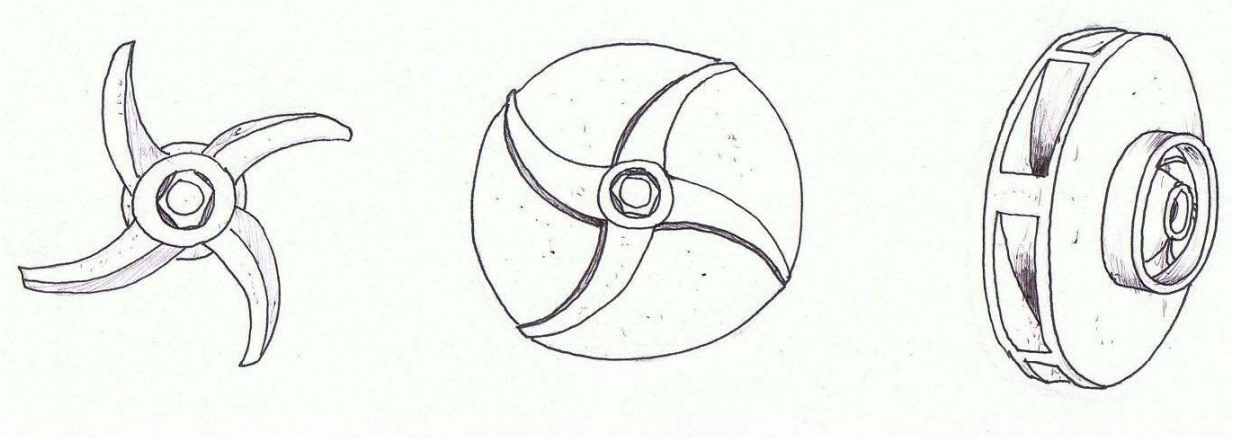
- ✓ can be up to 97% efficient.

- ✓ have good self-priming capabilities.

- ✓ can handle highly viscous liquids.
- ✓ are available for industrial, chemical and hygienic applications.
- ✓ cause a pulsating flow that may cause water hammer.

Vapor Pressure and Cavitation
Cavitation is the formation and then immediate implosion of cavities in a liquid – i.e. small liquid-free zones ("bubbles") – that are the consequence of forces acting upon the liquid. It usually occurs when a liquid is subjected to rapid changes of pressure that cause the formation of cavities where the pressure is relatively low.

Cavitation is a significant cause of wear in some engineering contexts. When entering high pressure areas, cavitation bubbles that implode on a metal surface cause cyclic stress. These results in surface fatigue of the metal causing a type of wear also called "cavitation". The most common examples of this kind of wear are pump impellers and bends when a sudden change in the direction of liquid occurs. Cavitation is usually divided into two classes of behavior: inertial (or transient) cavitation and non-inertial cavitation.



IMPELLER TYPES

Inertial Cavitation
Inertial cavitation is the process where a void or bubble in a liquid rapidly collapses, producing a shock wave. Inertial cavitation occurs in nature in the strikes of mantis shrimps and pistol shrimps, as well as in the vascular tissues of plants. In man-made objects, it can occur in control valves, pumps, propellers and impellers.

Non-inertial Cavitation
Non-inertial cavitation is the process in which a bubble in a fluid is forced to oscillate in size or shape due to some form of energy input, such as an acoustic field. Such cavitation is often employed in ultrasonic cleaning baths and can also be observed in pumps, propellers, etc. Since the shock waves formed by cavitation are strong enough to significantly damage moving parts, cavitation is usually an undesirable phenomenon. It is specifically avoided in the design of machines such as turbines or propellers, and eliminating cavitation is a major field in the study of fluid dynamics.

To understand Cavitation, you must first understand vapor pressure. Vapor pressure is the pressure required to boil a liquid at a given temperature. Soda water is a good example of a high vapor pressure liquid. Even at room temperature the carbon dioxide entrained in the soda is released. In a closed container, the soda is pressurized, keeping the vapor entrained.

Temperature affects vapor pressure as well, raises the water's temperature to 212°F and the vapors are released because at that increased temperature the vapor pressure is greater than the atmospheric pressure.

Pump cavitation occurs when the pressure in the pump inlet drops below the vapor pressure of the liquid. Vapor bubbles form at the inlet of the pump and are moved to the discharge of the pump where they collapse, often taking small pieces of the pump with them. Cavitation is often characterized by:

- ✓ Loud noise often described as a grinding or "marbles" in the pump.
- ✓ Loss of capacity (bubbles are now taking up space where liquid should be).
- ✓ Pitting damage to parts as material is removed by the collapsing bubbles.

Noise is a nuisance and lower flows will slow your process, but pitting damage will ultimately decrease the life of the pump.

In general, cavitation performance is related to some "critical" value:
NPSHA (=available) > NPSHc or NPSHR (=critical or required)
Typical "critical" characteristics identified for centrifugal pumps:
• Incipient cavitation (NPSHi)
• Developed cavitation causing 3% head drop (NPSH3%)
• Developed cavitation causing complete head breakdown(vapor lock).

Choice of NPSHR is rather arbitrary, but usually NPSHR=NPSH3%
Alternative choices:
• NPSHR=NPSH1% or NPSHR=NPSH5%
• NPSHR=NPSHi (cavitation free operation)

Cavitation causes or may cause:
• Performance loss (head drop)
• Material damage (cavitation erosion)
• Vibrations
• Noise
• Vapor lock (if suction pressure drops below break-off value)

The definition of NPSHA is simple: Static head + surface pressure head - the vapor pressure of your product - the friction losses in the piping, valves and fittings. But to really understand it, you first have to understand a couple of other concepts:

- ✓ Cavitation is what net positive suction head (NPSH) is all about, so you need to know a little about cavitation.
- ✓ Vapor Pressure is another term we will be using. The product's vapor pressure varies with the fluid's temperature.
- ✓ Specific gravity play an important part in all calculations involving liquid. You have to be familiar with the term.
- ✓ You have to be able to read a pump curve to learn the N.P.S.H. required for your pump.
- ✓ You need to understand how the liquid's velocity affects its pressure or head.
- ✓ It is important to understand why we use the term Head instead of Pressure when we make our calculations.
- ✓ Head loss is an awkward term, but you will need to understand it.

You will have to be able to calculate the head loss through piping, valves and fittings.

- ✓ You must know the difference between gage pressure and absolute pressure.
- ✓ Vacuum is often a part of the calculations, so you are going to have to be familiar with the terms we use to describe vacuum.

Let's look at each of these concepts in a little more detail:

- ✓ Cavitation means cavities or holes in liquid. Another name for a hole in a liquid is a bubble, so cavitation is all about bubbles forming and collapsing.
- ✓ Bubbles take up space so the capacity of our pump drops.
- ✓ Collapsing bubbles can damage the impeller and volute. This makes cavitation a problem for both the pump and the mechanical seal.
- ✓ Vapor pressure is about liquids boiling. If I asked you, "at what temperature does water boil?" You could say 212° F. or 100° C., but that is only true at atmospheric pressure. Every product will boil (make bubbles) at some combination of pressure and temperature. If you know the temperature of your product you need to know its vapor pressure to prevent boiling and the formation of bubbles. In the charts section of this web site you will find a vapor pressure chart for several common liquids.

- ✓ Specific gravity is about the weight of the fluid. Using 4°C (39° F) as our temperature standard we assign fresh water a value of one. If the fluid floats on this fresh water it has a specific gravity is less than one. If the fluid sinks in this water the specific gravity of the fluid is greater than one.

- ✓ Look at any pump curve and make sure you can locate the values for head, capacity, best efficiency point (B.E.P.), efficiency, net positive suction head (NPSH), and horse power required. If you cannot do this, have someone show you where they are located.

- ✓ Liquid velocity is another important concept. As a liquid's velocity increases, its pressure (90° to the flow) decreases. If the velocity decreases the pressure increases. The rule is : velocity times pressure must remain a constant.

- ✓ "Head" is the term we use instead of pressure. The pump will pump any liquid to a given height or head depending upon the diameter and speed of the impeller. The amount of pressure you get depends upon the weight (specific gravity) of the liquid. The pump manufacturer does not know what liquid the pump will be pumping so he gives you only the head that the pump will generate. You have to figure out the pressure using a formula described later on in this paper.

- ✓ Head (feet) is a convenient term because when combined with capacity (gallons or pounds per minute) you come up with the conversion for horsepower (foot pounds per minute).

- ✓ "Head loss through the piping, valves and fittings" is another term we will be using. Pressure drop is a more comfortable term for most people, but the term "pressure" is not used in most pump calculations so you could substitute the term "head drop" or "loss of head" in the system. To calculate this loss you will need to be able to read charts like those you will find in the "charts you can use" section in the home page of this web site. They are labeled Friction loss for water and Resistance coefficients for valves and fittings.

- ✓ Gage and absolute pressure. Add atmospheric pressure to the gage pressure and you get absolute pressure.

- ✓ Vacuum is a pressure less than atmospheric. At sea level atmospheric pressure is 14.7 psi. (760 mm of Mercury). Vacuum gages are normally calibrated in inches or millimeters of mercury.

To calculate the net positive suction head (NPSH) of your pump and determine if you are going to have a cavitation problem, you will need access to several additional pieces of information:

- ✓ The curve for your pump. This pump curve is supplied by the pump manufacturer. Someone in your plant should have a copy. The curve is going to show you the Net Positive Suction Head (NPSH) required for your pump at a given capacity. Each pump is different so make sure you have the correct pump curve and use the numbers for the impeller diameter on your pump. Keep in mind that this NPSH required was for cold, fresh water.

- ✓ A chart or some type of publication that will give you the vapor pressure of the fluid you are pumping.

- ✓ If you would like to be a little more exact, you can use a chart to show the possible reduction in NPSH required if you are pumping hot water or light hydrocarbons.

- ✓ You need to know the specific gravity of your fluid. Keep in mind that the number is temperature sensitive. You can get this number from a published chart, ask some knowledgeable person at your plant, or take a reading on the fluid using a hydrometer.

- ✓ Charts showing the head loss through the size of piping you are using between the source and the suction eye of your pump. You will also need charts to calculate the loss in any fittings, valves, or other hardware that might have been installed in the suction piping.

- ✓ Is the tank you are pumping from at atmospheric pressure or is it pressurized in some manner? Maybe it is under a vacuum ?

- ✓ You need to know the atmospheric pressure at the time you are making your calculation. We all know atmospheric pressure changes throughout the day, but you have to start somewhere.

The formulas for converting pressure to head and head back to pressure in the imperial system are as follows:

o sg. = specific gravity
o pressure = pounds per square inch
o head = feet
You also need to know the formulas that show you how to convert vacuum readings to feet of head. Here are a few of them:

To convert surface pressure to feet of liquid; use one of the following formulas:

- ✓ Inches of mercury x 1.133 / specific gravity = feet of liquid

- ✓ Pounds per square inch x 2.31 / specific gravity = feet of liquid

- ✓ Millimeters of mercury / (22.4 x specific gravity) = feet of liquid

There are different ways to think about net positive suction head (NPSH) but they all have two terms in common.

- ✓ NPSHA (net positive suction head available)
- ✓ NPSHR (net positive suction head required)

NPSHR (net positive suction head required) is defined as the NPSH at which the pump total head (first stage head in multi stage pumps) has decreased by three percent (3%) due to low suction head and resultant cavitation within the pump. This number is shown on your pump curve, but it is going to be too low if you are pumping hydrocarbon liquids or hot water.

Cavitation begins as small harmless bubbles before you get any indication of loss of head or capacity. This is called the point of incipient cavitation. Testing has shown that it takes from two to twenty times the NPSHR (net positive suction head required) to fully suppress incipient cavitation, depending on the impeller shape (specific speed number) and operating conditions. To stop a product from vaporizing or boiling at the low pressure side of the pump the NPSHA (net positive suction head available) must be equal to or greater than the NPSHR (net positive suction head required).

Priming a Pump

Liquid and slurry pumps can lose prime and this will require the pump to be primed by adding liquid to the pump and inlet pipes to get the pump started. Loss of "prime" is usually due to ingestion of air into the pump. The clearances and displacement ratios in pumps used for liquids and other more viscous fluids cannot displace the air due to its lower density.

Understanding Progressing Cavity Pump Theory

Progressing cavity pumps (PCPs) are a special type of rotary positive displacement pump where the produced fluid is displaced axially at a constant rate. This characteristic enables progressing cavity pumps to produce viscous, abrasive, multiphase and gaseous fluids and slurries over a wide range of flow rates and differential pressures. Progressing cavity pumps are comprised of two helicoidal gears (rotor and stator), where the rotor is positioned inside the stator. The combination of rotational movement and geometry of the rotor inside the stator results in the formation of cavities that move axially from pump suction to pump discharge.

Rotors are typically machined from high-strength steel and then coated with a wear resistant material to resist abrasion and reduce stator/rotor friction. Stators consist of steel tubular with an elastomer core bonded to the steel. The elastomer is molded into the shape of an internal helix to match the rotor.

In operation progressive cavity pumps are fundamentally fixed flow rate pumps, like piston pumps and peristaltic pumps, and this type of pump needs a fundamentally different understanding to the types of pumps to which people are more commonly first introduced, namely ones that can be thought of as generating pressure. This can lead to the mistaken assumption that all pumps can have their flow rates adjusted by using a

valve attached to their outlet, but with this type of pump this assumption is a problem, since such a valve will have practically no effect on the flow rate and completely closing it will involve very high pressures being generated. To prevent this, pumps are often fitted with cut-off pressure switches, burst disks (deliberately weak and easily replaced), or a bypass pipe that allows a variable amount a fluid to return to the inlet. With a bypass fitted, a fixed flow rate pump is effectively converted to a fixed pressure one.

At the points where the rotor touches the stator, the surfaces are generally traveling transversely, so small areas of sliding contact occur. These areas need to be lubricated by the fluid being pumped (Hydrodynamic lubrication). This can mean that more torque is required for starting, and if allowed to operate without fluid, called 'run dry', rapid deterioration of the stator can result. While progressive cavity pumps offer long life and reliable service transporting thick or lumpy

Helical Rotor and a Twin Helix
The progressive cavity pump consists of a helical rotor and a twin helix, twice the wavelength and double the diameter helical hole in a rubber stator. The rotor seals tightly against the rubber stator as it rotates, forming a set of fixed-size cavities in between. The cavities move when the rotor is rotated but their shape or volume does not change. The pumped material is moved inside the cavities.

The principle of this pumping technique is frequently misunderstood. Often it is believed to occur due to a dynamic effect caused by drag, or friction against the moving teeth of the screw rotor. In reality it is due to the sealed cavities, like a piston pump, and so has similar operational characteristics, such as being able to pump at extremely low rates, even to high pressure, revealing the effect to be purely positive displacement.

At a high enough pressure the sliding seals between cavities will leak some fluid rather than pumping it, so when pumping against high pressures a longer pump with more cavities is more effective, since each seal has only to deal with the pressure difference between adjacent cavities. Pumps with between two and a dozen (or so) cavities exist.

When the rotor is rotated, it rolls around the inside surface of the hole. The motion of the rotor is the same as the smaller gears of a planetary gears system. As the rotor simultaneously rotates and moves around, the combined motion of the eccentrically mounted drive shaft is in the form of a hypocycloid. In the typical case of single-helix rotor and double-helix stator, the hypocycloid is just a straight line. The rotor must be driven through a set of universal joints or other mechanisms to allow for the movement.

The rotor takes a form similar to a corkscrew, and this, combined with the off-center rotary motion, leads to the alternative name: eccentric screw pump.
Different rotor shapes and rotor/stator pitch ratios exist, but are specialized in that they don't generally allow complete sealing, so reducing low speed pressure and flow rate linearity, but improving actual flow rates, for a given pump size, and/or the pump's solids handling ability

Specific designs involve the rotor of the pump being made of a steel, coated with a smooth hard surface, normally chromium, with the body (the stator) made of a molded elastomer inside a metal tube body. The elastomer core of the stator forms the required complex cavities. The rotor is held against the inside surface of the stator by angled link

arms, bearings (immersed in the fluid) allowing it to roll around the inner surface (un-driven).

Elastomer
Elastomer is used for the stator to simplify the creation of the complex internal shape, created by means of casting, which also improves the quality and longevity of the seals by progressively swelling due to absorption of water and/or other common constituents of pumped fluids. Elastomer/pumped fluid compatibility will thus need to be taken into account. Two common designs of stator are the "equal-walled" and the "unequal-walled". The latter, having greater elastomer wall thickness at the peaks allows larger-sized solids to pass through because of its increased ability to distort under pressure. The former have a constant elastomer wall thickness and therefore exceed in most other aspects such as pressure per stage, precision, heat transfer, wear and weight. They are more expensive due to the complex shape of the outer tube.

Cavities are created by the geometry of the rotor and stator where the stator has one more lobe than the rotor. The cavities are moved axially along the pump by the rotating motion of the rotor. The motion of the rotor is a combination of a clockwise rotation of the rotor along its own axis and a counterclockwise rotation of the rotor eccentrically about the axis of the stator. Because the volume of each cavity remains constant throughout the process, the pump delivers a uniform non-pulsating flow. The total pressure capability of the pump is determined by the maximum pressure that can be generated within each cavity times the total number of cavities.

PC pumps are manufactured with a variety of stator/rotor tooth combinations. Typically artificial lift applications use a two-tooth stator and a single tooth rotor pump referred to as single-lobe pump. Higher stator/rotor tooth combinations, such as 3/2, are used to achieve higher volumetric and lift capacity although with higher torque requirements.

Understanding Pump NPSH
NPSH is an initialism for Net Positive Suction Head. In any cross-section of a generic hydraulic circuit, the NPSH parameter shows the difference between the actual pressure of a liquid in a pipeline and the liquid's vapor pressure at a given temperature. NPSH is an important parameter to take into account when designing a circuit: whenever the liquid pressure drops below the vapor pressure, liquid boiling occurs, and the final effect will be cavitation: vapor bubbles may reduce or stop the liquid flow, as well as damage the system.

Centrifugal pumps are particularly vulnerable especially when pumping heated solution near the vapor pressure, whereas positive displacement pumps are less affected by cavitation, as they are better able to pump two-phase flow (the mixture of gas and liquid), however, the resultant flow rate of the pump will be diminished because of the gas volumetrically displacing a disproportion of liquid. Careful design is required to pump high temperature liquids with a centrifugal pump when the liquid is near its boiling point.
The violent collapse of the cavitation bubble creates a shock wave that can literally carve material from internal pump components (usually the leading edge of the impeller) and creates noise often described as "pumping gravel". Additionally, the inevitable increase in vibration can cause other mechanical faults in the pump and associated equipment.

A somewhat simpler informal way to understand NPSH…

Fluid can be pushed down a pipe with a great deal of force. The only limit is the ability of the pipe to withstand the pressure. However, a liquid cannot be pulled up a pipe with much force because bubbles are created as the liquid evaporates into a gas. The greater the vacuum created, the larger the bubble, so no more liquid will flow into the pump. Rather than thinking in terms of the pump's ability to pull the fluid, the flow is limited by the ability of gravity and air pressure to push the fluid into the pump. The atmosphere pushes down on the fluid, and if the pump is below the tank, the weight of the fluid from gravity above the pump inlet also helps. Until the fluid reaches the pump, those are the only two forces providing the push. Friction loss and vapor pressure must also be considered. Friction loss limits the ability of gravity and air pressure to push the water toward the pump at high speed. Vapor pressure refers to the point at which bubbles form in the liquid. NPSH is a measure of how much spare pull you have before the bubbles form.

Some helpful information regarding atmospheric pressure; Atmospheric pressure is always naturally occurring and is always around us. At sea level, it equates to 101.325 kPa or approximately 14 Psi OR 10 meters of liquid pressure head. As we move higher up mountains, the air gets thinner and the atmospheric pressure reduces.
This should be taken into account when designing pumping systems. The reason there is atmospheric pressure is simply due to earth's gravity and its position in our solar system. It is a natural phenomenon and we are very lucky to have it as water wells and bores with shallow aquifers allow us to use this atmospheric pressure to our advantage.

We all know that pressure gauges exist on pumping systems and other machines to give us an indication of what performances are being achieved. We also use known pressures versus known performance in order to create a reference for system designs. An example would be an experienced pump technician or plumber knowing that a pressure of between 300 kPa and 500 kPa will provide adequate and comfortable pressure for household use.

A typical pressure gauge reads what is known as 'Gage Pressure,' or pressure relative to atmospheric pressure. An 'Absolute Pressure' gauge displays atmospheric pressure (typically 100 kPa or 14 psi or 10 meters of liquid pressure head) before any system had been connected. Manufacturers set typical gage pressure gauges to read ZERO at sea level as a standard, assuming designers will make allowances for the atmospheric pressure calculations themselves. Knowing this simple fact can make NPSH easier to understand.

If we now know that there is 100 kPa or 10 meters of head pressure, plus or minus whatever the gage pressure gauge shows, then we can safely see that this gives us an instant advantage of 10 meters of head pressure at sea level. This means we can borrow against this and drop a maximum of 10 meters into or under the ground (or below sea level) reducing the gauge to zero and still get natural 'push' into our pump. Great for wells and bores with shallow aquifers within this depth! It is important to note that to get to exactly 10 meters may be difficult, but with the correct pipework and system design, it is possible to get very close.

Once NPSH is fully understood, sizing and controlling pumps and pumping machines is a much simpler task.

NPSH is the liquid suction force at the intake of a pump. In other words, the force of a liquid naturally “pushing” into a pump from gravity pressure plus liquid head pressure only - into a single pump intake.

This means;
NPSH = the net (left over) positive pressure of suction force into a pump intake after friction loss has occurred. Liquid head height or liquid head pressure + gravity pressure, minus friction loss, leaves a net head pressure of force into the pump.

If we want to pump some amount of liquid, we have to ensure that this liquid can reach the center line of the suction point of the pump. NPSH represents the head (pressure and gravity head) of liquid in the suction line of the pump that will overcome the friction along the suction line.

NPSHR is the amount of liquid pressure required at the intake port of a pre-designed and manufactured pump. This is known as NPSHR (Net Positive Suction Head Required). The pump manufacturer will usually clearly have a NPSH curve to assist you in the correct installation.

NPSHA is the amount (A = available) to the pump intake after pipe friction losses and head pressures have been taken into account.

The reason for this requirement?
When the pump is receiving liquid at intake port and the impeller is pushing the liquid out the discharge port, they are effectively trying to tear each other apart because the pump is changing the liquid movement by a pressure increase at the impeller vanes, (general pump installations). Insufficient NPSHR will cause a low or near-vacuum pressure (negative NPSHA) to exist at the pump intake. This will cause the liquid to boil and cause cavitation, and the pump will not receive the liquid fast enough because it will be attempting to pump vapor. Cavitation will lower pump performance and damage pump internals.

At low temperatures the liquid can "hold together" (remain fluid) relatively easily, hence a lower NPSH requirement. However at higher temperatures, the higher vapor pressure starts the boiling process much quicker, hence a high NPSH requirement.

- ✓ Water will boil at lower temperatures under lower pressures. Conversely its boiling point is higher at higher pressures.

- ✓ Water boils at 100 degrees Celsius at sea level and an atmospheric pressure of 1 bar.

- ✓ Vapor Pressure is the pressure of a gas in equilibrium with its liquid phase at a given temperature. If the vapor pressure at a given temperature is greater than the pressure of the atmosphere above the liquid, then the liquid will boil. (This is why water boils at a lower temperature high in the mountains).

- ✓ At normal atmospheric pressure minus 5 psi (or -0.35 bar) water will boil at 89 degrees Celsius.

- ✓ At normal atmospheric pressure minus 10 psi (or -0.7 bar) water will boil at 69 degrees Celsius.

- ✓ At a positive pressure of +12 psi or +0.82 bar above atmospheric, water will boil at 118 degrees Celsius.

- ✓ Liquid temperature greatly affects NPSH and must be taken into account when expensive installations are being designed.

- ✓ A pump designed with a NPSHR suitable for cold water may cavitate when pumping hot water

Understanding Pump Vapor Pressure and Temperature
The boiling point is the temperature at which the liquid changes to the gaseous state. This point depends on external pressure. The normal boiling point is defined when the external pressure over the liquid = 1 atm.

Think about this:
What is the boiling point of water? What does it depend on?

Water boils at 100 degrees C at one atm external pressure (sea level). As the pressure is lowered, the boiling point is reduced.

Also, as the external pressure drops, the temperature where the vapor pressure = the external pressure, is lower.

The table shows the relationship between vapor pressure and temperature. Vapor pressure increases with an increase in temperature.

Temperature (°C)	Vapor Pressure (Torr)
100	760
50	93
20	17.5
0	5.5 (sublimation from ice)

Critical Temperature
Is there a point at which the gas can become a vapor and condense when the pressure increases?

Let's use Boyle's Law to explain.
Boyle's Law states that for a fixed amount of gas (n) at a constant temperature (T), the pressure (P) is inversely proportional to its volume (V). If the volume of the gas decreases, the pressure of the gas increases proportionally. This holds true because of the compressibility property of gases. But what if the volume of a gas were to decrease and in the process the gas condenses into liquid droplets? In such a situation, the saturated vapor pressure of the gas has been reached and the gas is now considered a vapor.

Understanding Pump Viscosity

When to use a centrifugal or a Positive Displacement pump ("PD Pump") is not always a clear choice. To make a good choice between these pump types it is important to understand that these two types of pumps behave very differently.

First let's examine the density of the substance to be pumped. The density of a substance is defined as its mass per unit volume, but here on the earth's surface, we can substitute weight for mass. At 39-deg F (4-deg C), water has a density of 8.34 pounds per gallon or 62.43 pounds per cubic foot. In the metric system its density is one gram per cubic centimeter, or 1,000-kg per cubic meter.

Specific Gravity
The term specific gravity compares the density of some substance to the density of water. Since specific gravity is the ratio of those densities, the units of measure cancel themselves, and we end up with a dimensionless number that is the same for all systems of measure. Therefore, the specific gravity of water is 1— regardless of the measurement system. Specific gravity is important when sizing a centrifugal pump because it is indicative of the weight of the fluid and its weight will have a direct effect on the amount of work performed by the pump. One of the beauties of the centrifugal pump is that the head (in feet) and flow it produces has nothing to do with the weight of the liquid. It is all about the velocity that is added by the impeller.

The simplest way to prove the validity of this statement is to use the falling body equation:
$v2 = 2gh$

Where:
v = Velocity
g = The universal gravitational constant
h = height.

This equation will predict the final velocity some object will attain when falling from some height (ignoring friction of course). When rearranged, it takes the form of $h = v^2/2g$ and predicts the maximum height an object can attain based on its initial velocity. The final velocity attained by a falling object is actually the same as the initial velocity required for it to rise to the same height from which it fell.

When this equation is applied to a centrifugal pump, h becomes the maximum theoretical head that it can produce. As the equation illustrates, that head depends upon

the exit velocity of the liquid from the impeller vanes and the effect of gravity; it has absolutely nothing to do with the weight of the liquid.

The weight of the liquid does affect the amount of work done by a pump and, therefore, the HP required. A good way to understand the impact of liquid weight is to convert flow in GPM and head in feet into units of work. The equation below performs this conversion.

(gpm X 8.34 lb/gal X h) = w

Here the flow is multiplied by the weight of a gallon of water and then multiplied by the head in feet. The result is the work performed in ft-lb/minute. The equation shows us that the amount of work done by a centrifugal pump is directly proportional to the weight of the pumped liquid. If you divide w by 33,000, the result is the HP required at that particular point of flow and head. The downward sloping curve in the upper portion of the graph is the H/Q curve and the red, blue and green curves are the horsepower curves for three different liquids. The scale of the Y axis is both head and horsepower. The blue curve shows the HP required for water (SG=1). The red and green curves show the HP required to pump sugar syrup (SG=1.29) and gasoline (SG=0.71). If you analyze the three HP curves at each flow point, you will see that the increase or decrease is directly proportional to the SG of that particular liquid.

As long as the viscosity of a liquid is similar to that of water, its specific gravity will have no effect on pump performance. It will, however, directly affect the input power required to pump that particular liquid. The equation below can be used to compute the horsepower required to pump liquids of varying specific gravities (where BHP is brake horsepower, Q is flow in GPM, H is head in feet, SG is specific gravity and Eff is the hydraulic efficiency of the pump). It assumes a viscosity similar to that of water.

BHP = (Q x H x SG) / (3960 x Eff)

SG can also have an effect on the onset of cavitation in a particular pump. Heavier liquids cause a proportional increase in a pump's suction energy and those with a high suction energy level are more likely to experience cavitation damage.

Understanding Pump Friction Loss

To optimize a fluid piping system, it is important to have a clear understanding of how the various system items interact. Regardless of the methods used to gain a thorough picture of piping system operations, a variety of calculations must be performed. Among the formulas are the Bernoulli equation to calculate the pressure in the system, and the Darcy-Weisbach equation, which is commonly used to calculate head loss in a pipe run. The Bernoulli Equation is a way of expressing the total energy of fluid as it flows through a pipe run

The Piping System

A piping system is configured of individual pipe runs connected in series and parallel combinations with pumps, control valves, flowmeters and components. It is essential to recognize how these unique elements interact and work together as a system. There are both graphical and analytical methods that provide an understanding of how the various items interact as a total system. The head loss is calculated using the graphical method for a variety of flow rates for each pipe run. The results can be read off the graph after

the information is plotted. Using the analytical method, the results are calculated directly, which eliminates the need for further graphics.

In fluid dynamics, the Darcy–Weisbach equation is a phenomenological equation, which relates the head loss — or pressure loss — due to friction along a given length of pipe to the average velocity of the fluid flow. The equation is named after Henry Darcy and Julius Weisbach.

The Darcy–Weisbach equation contains a dimensionless friction factor, known as the Darcy friction factor. This is also called the Darcy–Weisbach friction factor or Moody friction factor. The Darcy friction factor is four times the Fanning friction factor, with which it should not be confused.

Head Loss Formula
Head loss can be calculated with

$$h_f = f_D \cdot \frac{L}{D} \cdot \frac{V^2}{2g}$$

where

- *hf* is the head loss due to friction (SI units: m);
- L is the length of the pipe (m);
- D is the hydraulic diameter of the pipe (for a pipe of circular section, this equals the internal diameter of the pipe) (m);
- *V* is the average velocity of the fluid flow, equal to the volumetric flow rate per unit cross-sectional wetted area (m/s);
- *g* is the local acceleration due to gravity (m/s^2);
- *fD* is a dimensionless coefficient called the Darcy friction factor. It can be found from a Moody diagram or more precisely by solving the Colebrook equation. Do not confuse this with the Fanning Friction factor, f.

However the establishment of the friction factors was still an unresolved issue which needed further work.

Darcy-Weisbach Formula
Flow of fluid through a pipe
The flow of liquid through a pipe is resisted by viscous shear stresses within the liquid and the turbulence that occurs along the internal walls of the pipe, created by the roughness of the pipe material. This resistance is usually known as pipe friction and is measured is feet or meters head of the fluid, thus the term head loss is also used to express the resistance to flow.

Many factors affect the head loss in pipes, the viscosity of the fluid being handled, the size of the pipes, the roughness of the internal surface of the pipes, the changes in elevations within the system and the length of travel of the fluid. The resistance through various valves and fittings will also contribute to the overall head loss. A method to model the resistances for valves and fittings is described elsewhere.

In a well-designed system the resistance through valves and fittings will be of minor significance to the overall head loss, many designers choose to ignore the head loss for valves and fittings at least in the initial stages of a design.

Much research has been carried out over many years and various formulas to calculate head loss have been developed based on experimental data. Among these is the Chézy formula which dealt with water flow in open channels. Using the concept of 'wetted perimeter' and the internal diameter of a pipe the Chézy formula could be adapted to estimate the head loss in a pipe, although the constant 'C' had to be determined experimentally.

The Darcy-Weisbach Equation
Weisbach first proposed the equation we now know as the Darcy-Weisbach formula or Darcy-Weisbach equation:

$hf = f (L/D) \times (v2/2g)$

where:
hf = head loss (m)
f = friction factor
L = length of pipe work (m)
d = inner diameter of pipe work (m)
v = velocity of fluid (m/s)
g = acceleration due to gravity (m/s^2)
or:
hf = head loss (ft)
f = friction factor
L = length of pipe work (ft)
d = inner diameter of pipe work (ft)
v = velocity of fluid (ft/s)
g = acceleration due to gravity (ft/s^2)

The Moody Chart
In 1944 LF Moody plotted the data from the Colebrook equation and this chart which is now known as 'The Moody Chart' or sometimes the Friction Factor Chart, enables a user to plot the Reynolds number and the Relative Roughness of the pipe and to establish a reasonably accurate value of the friction factor for turbulent flow conditions. The Moody Chart encouraged the use of the Darcy-Weisbach friction factor and this quickly became the method of choice for hydraulic engineers. Many forms of head loss calculator were developed to assist with the calculations, amongst these a round slide rule offered calculations for flow in pipes on one side and flow in open channels on the reverse side.

The development of the personnel computer from the 1980's onwards reduced the time needed to perform the friction factor and head loss calculations, which in turn has widened the use of the Darcy-Weisbach formula to the point that all other formula are now largely unused.

Pipe Runs
A piping system is composed primarily of individual pipe runs connecting all system elements together. Because a pipe run is the basic building block of a piping system, examine the losses associated with individual pipe runs when connected in series and

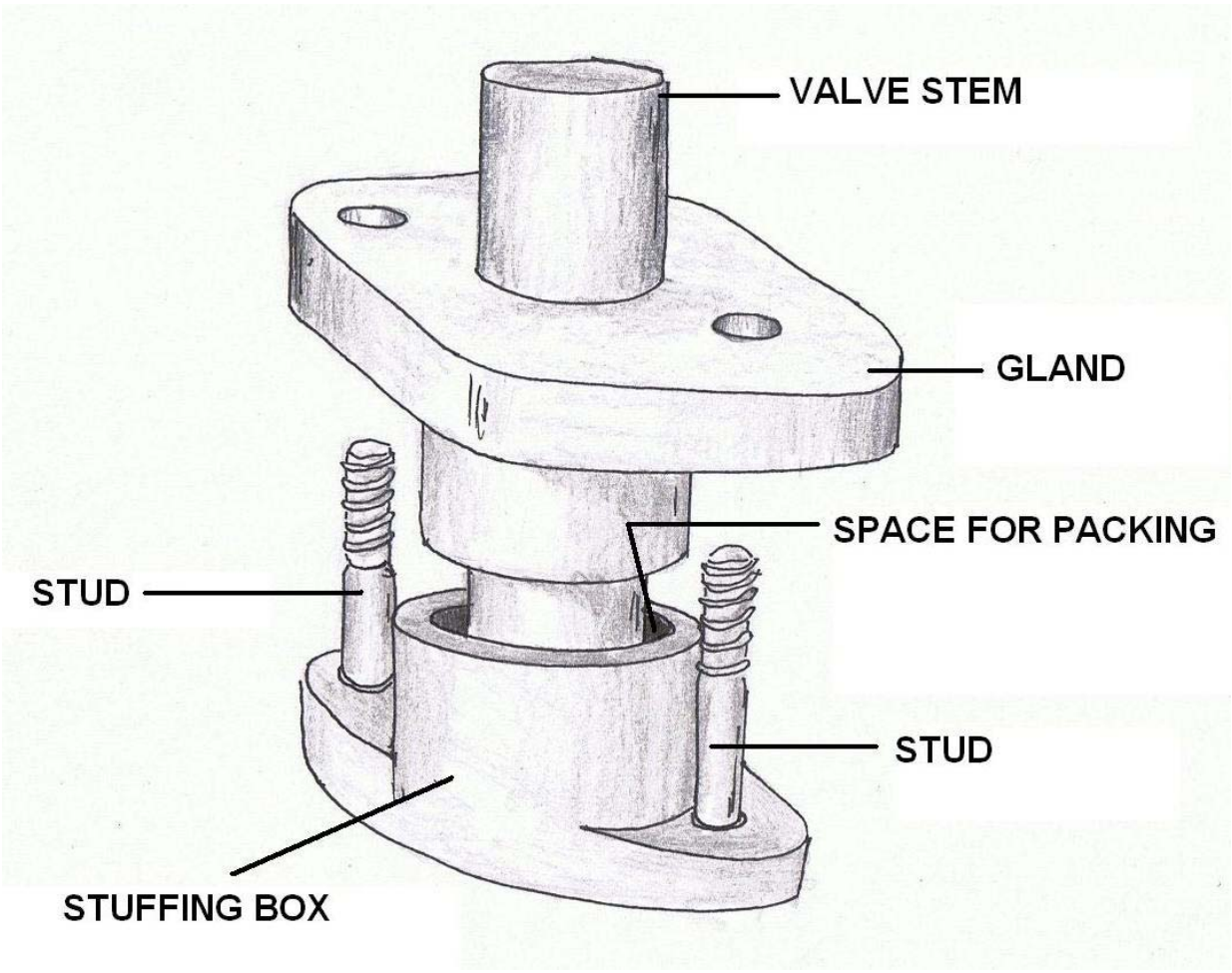
parallel configurations. The pipe head loss in a single pipe run can easily be calculated using the Darcy-Weisbach equation. Performing the head loss calculation for a range of expected flow rates helps to develop a curve showing the pipe run head loss for any flow rate within a defined range. The Bernoulli equation allows for calculation of pressure anywhere in the pipe run.

Multiple pipe runs connected end-to-end form a "series" of individual pipe runs. The flow rate through each pipe run in a series configuration is identical. As a result, the head loss for a series of pipe runs is simply the sum of the head losses for each of the individual pipe runs. When multiple pipe runs are placed in parallel, determining the head loss through them becomes more difficult because the flow is distributed through the various pipe runs. The head loss across the parallel paths can be calculated after determining the flow rate in each pipe run and the head loss across each pipe run in a parallel configuration.
A component-including filters, strainers, towers, columns and heat exchangers-is an item placed in a piping system that has a head loss for a given flow rate. The function describing the head loss across the component versus the flow rate is similar to that of the head loss through valves and fittings.

Pump Curves
A pump curve describes the operation of a pump for a range of flows at a defined speed. Many design elements affect the shape of the pump curve, and most of these cannot be changed by the user. As a result, centrifugal pumps are usually selected from the manufacturer's available designs to match the system requirements. An engineered or assembled-to-order pump can be specified, and the manufacturer can often provide a pump performance characteristic well suited to the specific application depending on the type of pump. Characteristics that can be changed by users to change the pump (performance) curve are the impeller diameter and the rotational speed. The pump curve change will cause the pump curve to intersect the system curve at a different rate of flow. When selected properly, the pump will operate near its best efficiency point (BEP). This relationship of speed change or diameter change is often referred to as the pump affinity rules.

Control valves are inserted into a piping system to regulate the rate of flow or pressure in the piping system. Remember, control valves control the flow by providing a variable hydraulic resistance between the upstream and downstream components in the system. In other words, the control valve does not change the basic shape of the system curve; it provides additional resistance to the system to enable the valve to control the flow.

System Curves
Pump and system curves can illustrate the basic interaction in the total system. Pump and system curves consist of a system curve showing the head required to pass a given flow rate through the piping system, and a pump curve superimposed on the system curve. The point where the system curve and the pump curve intersect is the balanced flow rate through the pump. In the absence of control valves, the system will operate at the intersection of the pump and system curves.



Installation of Standard Stuffing Boxes

1. Stuffing Box

A. Slide the stuffing box over the shaft and fit into place (be sure to include the o-ring or gasket below the stuffing box flange). Bolt securely in place using the studs and nuts provided.

2. Packing

A. Insert four packing rings, fitting ends together so they contact face to face on the cut end. Turn each cut piece 90O from the previous piece. Be sure each piece is set against the piece below it.

CAUTION

Do not tamp packing tight in the stuffing box. Excessive tamping will stop the flow of fluid through the packing. This will result in the destruction of the shaft area.

3. Packing Gland

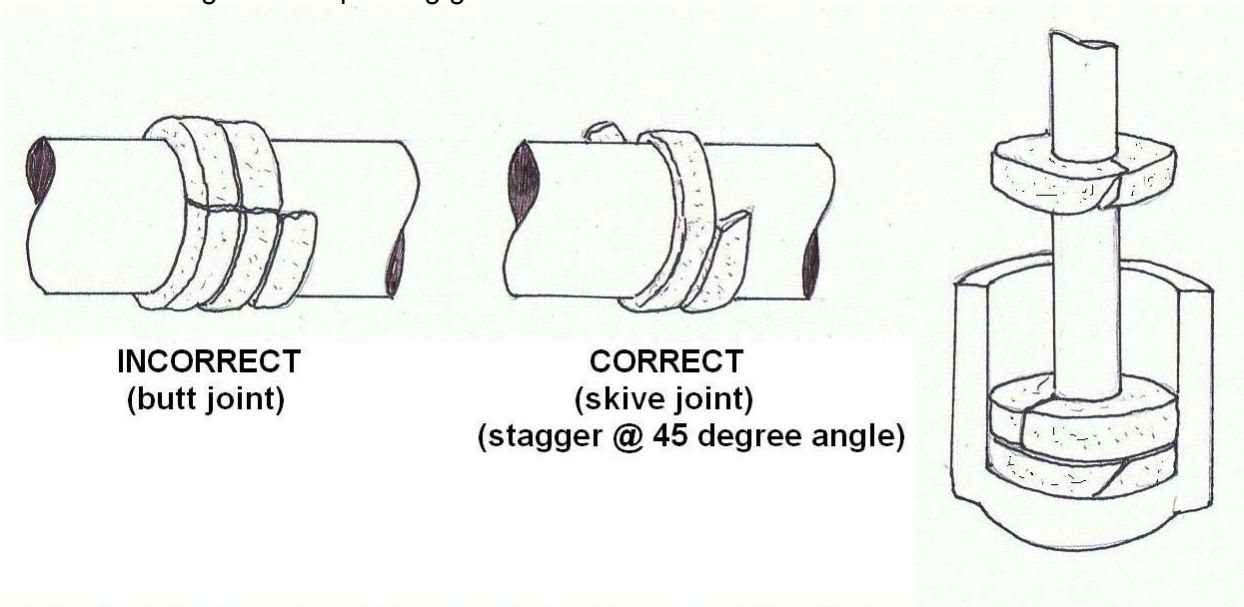
A. Thread the two studs in the threaded holes on top of the stuffing box. Insert the packing gland on top of the packing and pull snug (not tight). The packing gland nuts should be tightened together to keep equal pressure on the packing.

4. Slinger

A. Attach slinger above packing gland.

CAUTION
The stuffing box must be allowed to leak for proper operation. The proper amount of leakage can be determined by checking the temperature of the leakage. This should be cool or just lukewarm, not hot. Shutting off leakage flow from the packing will result in burned packing and a scored shaft.

Installation of Optional Stuffing Boxes
A. Stuffing Box
A. Slide the stuffing box over the shaft and fit into place (be sure to include the o-ring or gasket on the bottom
side of the stuffing box in the groove provided. Bolt securely in place using the studs and nuts.
B. Packing
a. Insert the lower lantern ring (threaded holes up) in bottom of box.
b. Insert three packing rings, fitting ends together so they contact face to face on an angle. Turn each cut piece 90^{O} from the previous piece. Be sure each piece is set against the piece below it.
c. Insert the second lantern ring (threaded holes up) on top of the packing. The lantern ring should be aligned with the grease port.
d. Insert three more packing rings on top of the lantern ring, as before.
e. Thread two studs into the holes on top of the stuffing box.
f. Insert the packing gland on top of packing, press down snug. The packing gland nuts should be tightened together to keep equal pressure on the packing.
The packing must be allowed to leak for proper operation. The proper amount of leakage can be determined by checking the temperature of the leakage. This should be cool or just lukewarm, not hot.
g. Insert the grease zerk and grease with a high quality grease.
C. If high pressure bypass is necessary, remove bypass plug. Install bypass line back to suction side of pump or drain.
D. Attach a slinger above packing gland.



Installation of Mechanical Seals

1. General Information
A. Study all instructions before installing.
2. Equipment Preparation
A. Assembled Pumps
a. The throttle bushing housing is shipped assembled on the pump but without the seal installed.
b. All faces of the mechanical seal housing and the throttle bushing housing must be free from dirt and rust.
B. Unassembled Pumps
a. The mechanical seal and throttle bushing housing are packaged in the box of small parts.
b. All faces of the mechanical seal housing, throttle bushing and head must be free from dirt and rust.
c. Install the o-ring or gasket in the throttle bushing housing, slide throttle bushing housing over shaft and seat it against the discharge head. Bolt securely in place using the studs & nuts provided.
2. Seal Installation
A. Before installation of Vertical Solid Shaft Motor (VSS) or Head Shaft in Vertical Hollow Shaft (VHS) Motor Installation.
B. Assure that shaft & seal housing are clean and free of machining and handling burrs
C. Set seal in place over pump shaft. Apply teflon tape to shaft treads and lubricate to ease seal into place.
D. Using fasteners provided, secure seal gland to seal housing
E. In the case of VSS Motor Installation
1. Install pump shaft key and coupling half
2. Install coupling spacer and run down to full shaft thread length
3. Affix key and Motor coupling half to VSS Motor
F. Set VSS or VHS Motor in place and bolt to Discharge Head
G. In the case of VSS Motor
1. Rotate coupling spacer in reverse direction to installation in order to elevate coupling spacer to the appropriate impeller adjustment if contact with motor coupling half.
2. Rotate motor to align motor half coupling holes with the holes in the pump coupling half.
3. Install and tighten coupling bolts (at which point pump shaft will be elevated to the appropriate impeller adjustment.
H. In the case of VHS Motor
1. Thread shaft coupling onto pump shaft.
2. Install motor (head) shaft through opening (quill) of motor (be careful not to impact coupling
threads).
3. Hold shaft coupling and thread motor shaft into coupling.
4. Rotate motor by cooling fins until the female key slot is in alignment with the motor (Head) shaft.
5. Install Gibb Key into slot presented by the motor (head) shaft and the motor coupling on the top end of the motor.
6. Install adjusting nut in top end of motor (Head) shaft.
7. Tighten adjusting nut until shaft and string is elevated to appropriate impeller adjustment.

8. Rotate adjustment nut until one of the 1/4” - 20unc holes and the motor coupling on the top end of the motor, and one of the 5/16” holes in the adjustment nut are in alignment. (Note: Rotate the adjustment in the direction that assures minimum vertical shaft movement.)
9. Install and tighten the 1/4” - 20unc bolt provided into the aligned bolt hole.
I. Tighten the 1/4” - 20unc Allen (grub) screws located on the mechanical seal, onto the top shaft.
J. Remove the 1/4” - 20unc beveled head machine screws and aluminum spacer clips and store these parts in a secure place for use upon removal of the mechanical seal.
3. Seal Removal
Reverse the above process.

Installation of Hollow Shaft Drivers
1. Clean driver mounting flange on discharge head and check for burrs or nicks on the register and mounting face. Oil lightly.
2. Remove driver clutch.
3. See No. 10 regarding the installation of motor guide bushing, if required.
4. Lift driver and clean mounting flange, checking for burrs and nicks.
5. Center motor over pump and rotate to align mounting holes.
6. Lower carefully into place making certain that the female register on the driver mates over the male register on the pump.
7. Bolt driver to discharge head.
8. Check driver manufacturer’s instruction manual for special instructions including lubrication instructions and follow all “start-up” directions.
9. Electric motors should be checked for rotation at this time. Make certain the driver clutch has been removed. Make electrical connections to the job motor and momentarily check rotation. DRIVER MUST ROTATE COUNTER CLOCKWISE
when looking down at the top end of the motor. To change the direction of rotation on a three phase motor, interchange any two line leads. To change direction of rotation on a two phase motor, interchange the leads of either phase.
10. Some electric motors will be supplied with a “lower guide bushing” which is installed at the bottom of the motor to stabilize the shaft at this point. Some motor manufacturers mount this guide bushing before shipping while others will ship the guide bushing with instructions for field mounting. Check the packing slip to see of a guide bushing is required,
if so, determine if the bushing is already mounted or not and proceed accordingly. Refer to the Motor Instruction Manual.
11. Install coupling on driver being careful that it fits properly.
12. At this point, if the pump is supplied with a two piece head shaft construction, attach the headshaft to the topshaft with a coupling and tighten the shafts (left hand threads).
13. Clean threads on top of headshaft and headshaft nut. Lubricate male threads lightly.
14. Install Gibb Key in coupling and shaft. This must be a sliding fit and may require filling and dressing. Do not force.
15. Thread adjusting nut down on shaft until it bears against coupling. (Threads on 1-11/16” and larger head shaft adjusting nuts are left-handed, all other are right-handed). Do not thread nut further at this time. See impeller adjusting instructions.

Understanding Centrifugal Pump

Centrifugal pumps, are a sub-class of dynamic axisymmetric work-absorbing turbomachinery. Centrifugal pumps are used to transport liquids/fluids by the conversion of the rotational kinetic energy to the hydro dynamics energy of the liquid flow. The rotational energy typically comes from an engine or electric motor or turbine. In the typical simple case, the fluid enters the pump impeller along or near to the rotating axis and is accelerated by the impeller, flowing radially outward into a diffuser or volute chamber (casing), from where it exits.

Common uses include water, sewage, petroleum and petrochemical pumping. The reverse function of the centrifugal pump is the water turbine that converts potential energy of water pressure into mechanical rotational energy.

The transfer of energy from the mechanical rotation of the impeller to the motion and pressure of the fluid is usually described in terms of centrifugal force, especially in older sources written before the modern concept of centrifugal force as a fictitious force in a rotating reference frame was well articulated. The concept of centrifugal force is not actually required to describe the action of the centrifugal pump.

In the modern centrifugal pump, most of the energy conversion is due to the outward force that curved impeller blades impart on the fluid. Invariably, some of the energy also pushes the fluid into a circular motion, and this circular motion can also convey some energy and increase the pressure at the outlet.

Modern sources say things like that the fluid "flows radially under centrifugal force", or "centrifugal force flings the liquid outward". Others counter that "there is no force at all, and a great deal of confused thinking." Some are more careful, attributing the outward force to the impeller, not to centrifugal force: "the impellers throw the water to the outside of the impeller case. This centrifugal action is what creates the pressure..." Even serious texts that explain the working of the pump without mention of centrifugal force introduce the pump as one in which "the mechanical energy is converted, into pressure energy by means of centrifugal force acting on the fluid."

A centrifugal pump is one of the simplest pieces of equipment in any process plant. Its purpose is to convert energy of a prime mover (an electric motor or turbine) first into velocity or kinetic energy and then into pressure energy of a fluid that is being pumped. The energy changes occur by virtue of two main parts of the pump, the impeller and the volute or diffuser. The impeller is the rotating part that converts driver energy into the kinetic energy. The volute or diffuser is the stationary part that converts the kinetic energy into pressure energy.
Note: All of the forms of energy involved in a liquid flow system are expressed in terms of feet of liquid i.e. head.

Generation of Centrifugal Force
The process liquid enters the suction nozzle and then into eye (center) of a revolving device known as an impeller. When the impeller rotates, it spins the liquid sitting in the cavities between the vanes outward and provides centrifugal acceleration. As liquid leaves the eye of the impeller a low-pressure area is created causing more liquid to flow toward the inlet. Because the impeller blades are curved, the fluid is pushed in a

tangential and radial direction by the centrifugal force. This force acting inside the pump is the same one that keeps water inside a bucket that is rotating at the end of a string.

Vertical Centrifugal Pumps
Vertical centrifugal pumps are also referred to as cantilever pumps. They utilize a unique shaft and bearing support configuration that allows the volute to hang in the sump while the bearings are outside of the sump. This style of pump uses no stuffing box to seal the shaft but instead utilizes a "throttle Bushing". A common application for this style of pump is in a parts washer.

Froth Pumps
In the mineral processing industry, or in the extraction of oils and, froth is generated to separate the rich minerals or bitumen from the sand and clays. Froth contains air that tends to block conventional pumps and cause loss of prime. The industry over the years has developed different ways to deal with this problem. One approach consists of using vertical pumps with a tank. Another approach is to build special pumps with an impeller capable of breaking the air bubbles. In the pulp and paper industry holes are drilled in the impeller. Air escapes to the back of the impeller and a special expeller discharges the air back to the suction tank. The impeller may also feature special small vanes between the primary vanes called split vanes or secondary vanes. Some pumps may feature a large eye, an inducer or recirculation of pressurized froth from the pump discharge back to the suction to break the bubbles.

Multistage Centrifugal Pumps
A centrifugal pump containing two or more impellers is called a multistage centrifugal pump. The impellers may be mounted on the same shaft or on different shafts. For higher pressures at the outlet impellers can be connected in series. For higher flow output impellers can be connected in parallel. All energy transferred to the fluid are derived from the mechanical energy driving the impeller.

Priming
Most centrifugal pumps are not self-priming. In other words, the pump casing must be filled with liquid before the pump is started, or the pump will not be able to function. If the pump casing becomes filled with vapors or gases, the pump impeller becomes gas-bound and incapable of pumping. To ensure that a centrifugal pump remains primed and does not become gas-bound, most centrifugal pumps are located below the level of the source from which the pump is to take its suction. The same effect can be gained by supplying liquid to the pump suction under pressure supplied by another pump placed in the suction line.

A centrifugal pump adds velocity to a liquid, but first it must get the liquid. As the centrifugal pump throws liquid out from the eye of the impeller, the volute design creates a low pressure area where the liquid used to be. At that point, either atmospheric pressure, gravity, or a combination of the two will fill up the low pressure area with either more liquid or additional air. The problem with centrifugal pumps is that a given impeller diameter and speed will throw all fluids (either a liquid or a gas) to the same height. Since air qualifies as a fluid it will throw air to the same height as water. That height is not enough to overcome atmospheric pressure, so the centrifugal pump has to have all of its air removed before it will pump a liquid, and that is what we mean by priming the pump.

There are several methods you can use to remove air from a centrifugal pump:

- ✓ You can fill the pump and suction piping with liquid and start all over again.
- ✓ You can attach a priming pump to the discharge side of the pump to remove any air in the pump and suction piping. Be sure this pump has a mechanical seal. You never want to use packing in a priming pump because air will leak into the stuffing box through the packing.
- ✓ Some people install a foot valve at the end of the suction piping to insure that the fluid will not drain from the pump and suction piping. These valves seldom work out because, like all check valves, they leak.

The self-priming pump will retain enough fluid when it stops, to start again without having to worry about re-priming. A toilet or sink trap performs a similar function when it retains liquid to prevent vapors and odors from coming into your house.

There are a couple of ways to do this:

- ✓ Change the volute and impeller casing so that it retains the liquid in a built in reservoir that is filled during the initial priming phase and retains this fluid when the pump completes its pumping task and shuts down. An internal recirculation port then connects the discharge of the pump back to the suction cavity allowing a continuous recirculation of liquid during the priming phase.
- ✓ Design a suction and discharge cavity above the centerline of the impeller eye insuring that the pump is always full of liquid.

Understanding Suction Lift

Suction lift deals with the maximum distance to the intake of a pump. Fire pumps and others may lift about 5' to 10' of suction. You must lower the pump continually towards the water to keep them pumping. This creates a water risk, and when they put it back in, it pumps for a while, and if it quits again, then the same process must be repeated until it is pumping properly. Pumps operating at a negative minimum inlet pressure are capable of creating a suction lift (non-self-priming). The suction capacity is approximately equal to the level of the negative minimum inlet pressure minus a 1 m safety factor.

NPSH is initialism for Net Positive Suction Head. In any cross-section of a generic hydraulic circuit, the NPSH parameter shows the difference between the actual pressure of a liquid in a pipeline and the liquid's vapor pressure at a given temperature.

NPSH is an important parameter to take into account when designing a circuit: whenever the liquid pressure drops below the vapor pressure, liquid boiling occurs, and the final effect will be cavitation: vapor bubbles may reduce or stop the liquid flow, as well as damage the system.

Centrifugal pumps are particularly vulnerable especially when pumping heated solution near the vapor pressure, whereas positive displacement pumps are less affected by cavitation, as they are better able to pump two-phase flow (the mixture of gas and liquid), however, the resultant flow rate of the pump will be diminished because of the gas volumetrically displacing a disproportion of liquid. Careful design is required to pump high temperature liquids with a centrifugal pump when the liquid is near its boiling point.

The violent collapse of the cavitation bubble creates a shock wave that can literally carve material from internal pump components (usually the leading edge of the impeller) and creates noise often described as "pumping gravel". Additionally, the inevitable increase in vibration can cause other mechanical faults in the pump and associated equipment.

$$NPSH = \frac{p_0 - p_v}{\rho g} + \Delta z - h_L$$

where $h_L$is the head loss between 0 and 1, $p_0$is the pressure at the water surface, $p_v$is the vapour pressure (saturation pressure) for the fluid at the temperature T_1at 1, $\Delta z$is the difference in height $z_1 - z_0$ from the water surface to the location 1, and $\rho$is the fluid density, assumed constant, and $g$is gravitational acceleration.

where is the head loss between 0 and 1, is the pressure at the water surface, is the vapor pressure (saturation pressure) for the fluid at the temperature at 1, is the difference in height (shown as H on the diagram) from the water surface to the location 1, and is the fluid density, assumed constant, and is gravitational acceleration.

Suction Limitations
Regardless of the extent of the vacuum, water can only be “lifted” a set distance or height due to it’s' vaporization pressure. As the pressure above the water is reduced, the water will tend to rise as a result of the atmospheric pressure, which is tending to push the water into the pump suction piping. The theoretical maximum suction lift for water is 33.9 feet. From a practical standpoint, in consideration of the friction loss of the piping, the altitude of the station, etc., the normal maximum lift for any pump is approximately 25 ft. However, it must be remembered that cavitation of the impeller increases as the suction lift increases , and therefore, the pump, where possible, should be located so that the suction line is submerged at all times.

Pumps lift water with the help of atmospheric pressure, then pressurize and discharge the water from the casing. The practical suction lift, at sea level is 25 feet. Most pump manufacturers will list this as the maximum suction lift. Static suction lift is the maximum distance from the water level, to the centerline of the impeller. The main type of pump used for suction lift is a vertical shaft turbine pump.

Suction lift exists when a liquid is taken from an open tank to an atmospheric tank where the liquid level is below the centerline of the pump suction.

The following relationships may help to better understand Suction Lift:
Total Dynamic Head = Total discharge head + Total Suction Lift
Total Suction Lift = static + friction

Depending on how the measurement is taken suction lift and head may also be referred to as static or dynamic. Static indicates the measurement does not take into account the friction caused by water moving through the hose or pipes. Dynamic indicates that losses due to friction are factored into the performance. The following terms are usually used when referring to lift or head.

Static Suction Lift - The vertical distance from the water line to the centerline of the impeller.

Static Discharge Head - The vertical distance from the discharge outlet to the point of discharge or liquid level when discharging into the bottom of a water tank.

Dynamic Suction Head - The Static Suction Lift plus the friction in the suction line. Also referred to as a Total Suction Head.

Dynamic Discharge Head - The Static Discharge Head plus the friction in the discharge line. Also referred to as Total Discharge Head.

Total Dynamic Head - The Dynamic Suction Head plus the Dynamic Discharge Head. Also referred to as Total Head.

Suction Lift Chart
The vertical distance that a pump may be placed above the water level (and be able to draw water) is determined by pump design and limits dictated by altitude. The chart below shows the absolute limits. The closer the pump is to the water level, the easier and quicker it will be to prime.

Suction Lift at Various Elevations

Altitude:	Suction Lift In Feet
Sea Level	25.0
2,000 ft.	22.0
4,000 ft.	19.5
6,000 ft.	17.3
8,000 ft.	15.5
10,000 ft.	14.3

Understanding Affinity Laws
The Affinity Laws
The affinity laws are used in hydraulics and HVAC to express the relationship between variables involved in pump or fan performance (such as head, volumetric flow rate, shaft speed) and power. They apply to pumps, fans, and hydraulic turbines. In these rotary implements, the affinity laws apply both to centrifugal and axial flows.

The affinity laws are useful as they allow prediction of the head discharge characteristic of a pump or fan from a known characteristic measured at a different speed or impeller diameter. The only requirement is that the two pumps or fans are dynamically similar, that is the ratios of the fluid forced are the same.

These laws assume that the pump/fan efficiency remains constant i.e. . When applied to pumps the laws work well for constant diameter variable speed case (Law 1) but are less accurate for constant speed variable impeller diameter case (Law 2).

Law 1a. Flow is proportional to shaft speed:

$$\frac{Q_1}{Q_2} = \left(\frac{N_1}{N_2}\right)$$

Law 1b. Pressure or Head is proportional to the square of shaft speed:

$$\frac{H_1}{H_2} = \left(\frac{N_1}{N_2}\right)^2$$

Law 1c. Power is proportional to the cube of shaft speed:

$$\frac{P_1}{P_2} = \left(\frac{N_1}{N_2}\right)^3$$

Law 2. With shaft speed (N) held constant:

Law 2a. Flow is proportional to impeller diameter:

$$\frac{Q_1}{Q_2} = \left(\frac{D_1}{D_2}\right)^3$$

Law 2b. Pressure or Head is proportional to the square of impeller diameter:

$$\frac{H_1}{H_2} = \left(\frac{D_1}{D_2}\right)^2$$

Law 2c. Power is proportional to the cube of impeller diameter:

$$\frac{P_1}{P_2} = \left(\frac{D_1}{D_2}\right)^5$$

where

- Q is the volumetric flow rate (e.g. CFM, GPM or L/s),
- D is the impeller diameter (e.g. in or mm),
- N is the shaft rotational speed (e.g. rpm),
- H is the pressure or head developed by the fan/pump (e.g. ft. or m), and
- P is the shaft power (e.g. W).

These laws assume that the pump/fan efficiency remains constant i.e. $\eta_1 = \eta_2$. When applied to pumps the laws work well for constant diameter variable speed case (Law 1) but are less accurate for constant speed variable impeller diameter case (Law 2).

Understanding Pump Performance

The formula for calculating NPSHA:
NPSHA
Term = HA ± HZ - HF + HV - HVP

The formula for calculating $NPSH_A$:

Term	Definition	Notes
H_A	The absolute pressure on the surface of the liquid in the supply tank	Typically atmospheric pressure (vented supply tank), but can be different for closed tanks. Don't forget that altitude affects atmospheric pressure (H_A in Denver, CO will be lower than in Miami, FL). Always positive (may be low, but even vacuum vessels are at a positive absolute pressure)
H_Z	The vertical distance between the surface of the liquid in the supply tank and the centerline of the pump	Can be positive when liquid level is above the centerline of the pump (called static head) Can be negative when liquid level is below the centerline of the pump (called suction lift) Always be sure to use the lowest liquid level allowed in the tank.
H_F	Friction losses in the suction piping	Piping and fittings act as a restriction, working against liquid as it flows towards the pump inlet.
H_V	Velocity head at the pump suction port	Often not included as it's normally quite small.
H_{VP}	Absolute vapor pressure of the liquid at the pumping temperature	Must be subtracted in the end to make sure that the inlet pressure stays above the vapor pressure. Remember, as temperature goes up, so does the vapor pressure.

Understanding D-C Motors

DC motors have been available for nearly 100 years. In fact the first electric motors were designed and built for operation from direct current power. AC motors are the basic prime movers for the fixed speed requirements of industry. Their basic simplicity, dependability and ruggedness make AC motors the natural choice for the vast majority of industrial drive applications.

An electric motor can be configured as a solenoid, a stepper motor or a rotational machine. This article covers the DC rotational machine. In all DC rotational machines, there are six components that comprise the electric motor: axle, rotor or armature, stator, commutator, field magnets and brushes.

In order to understand how a direct current (DC) electric motor operates, a few basic principles must be understood. Just as in Faraday's experiment, the DC motor works with magnetic fields and electrical current. Centuries ago it was discovered that a stone found in Asia, referred to as a lodestone, and had an unusual property that would transfer an invisible force to an iron object when the stone was rubbed against it. These lodestones were found to align with the earth's north-south axis when freely hanging on a string or floated on water, and this property aided early explorers in navigating around the earth.

It was understood later that this stone was a permanent magnet with a field that had two poles of opposite effect, referred to as north and south. The magnetic fields, just like electric charges, have forces that are opposite in their effects. Electric charges are either positive or negative, whereas magnetic fields have a north-south orientation. When magnetic fields are aligned at opposite or dissimilar poles, they'll exert considerable forces of attraction with one another, and when aligned at like or similar poles, they'll strongly repel one another.

The magnetic field will pull or put a force upon a ferrous (magnetic) material. If iron particles are sprinkled on a paper sheet over a permanent magnet, the alignment of the iron particles maps the magnetic field, which shows that this field leaves one pole and enters the other pole with the force field being unbroken. As with any kind of field (electric, magnetic or gravitational), the total quantity, or effect, of the field is referred to as the flux, while the push causing the flux to form in space is called a force. This magnetic force field is comprised of many lines of flux, all starting at one pole and returning to the other pole.

Modern Theory of Magnetism

The modern theory of magnetism states that a magnetic field is produced by an electric charge in motion. When an electric charge is in motion, the electrons orbiting the atom are forced to align and uniformly spin in the same direction. The more atoms uniformly spinning in the same direction, the stronger the force of the magnetic field. When billions of atoms have orbits spinning in the same direction and the material is capable of holding the atoms' orbits, a permanent magnet is created.

When two powerful permanent magnets are moved in close proximity to one another, it's evident that a very real force is exerted that can provide the potential for work to be done. For work to be accomplished, the relationship between the magnetic fields must be controlled properly. The trick here is to control the magnetic fields by a means other

than just using the permanent magnet. This can be accomplished by producing a magnetic field with an electrical conductor that has current flowing through it.

Nearly all electric motors exploit the use of a current-carrying conductor to create mechanical work. When current is flowing through a conductor and the electric charge is in motion, the electrons orbiting the atoms are forced to align and uniformly spin in the same direction. This creates a magnetic field that forms around the conductor. The larger the current flowing through the conductor, the more atoms are forced to align and rotate in a uniform direction.

This rotational alignment of the atoms increases the strength of the magnetic field. However, if one were to place a conductor with current flowing through it near a permanent magnet, he would be disappointed by how feeble this force is. What's needed is a way to amplify the magnetic force field. This is accomplished by taking the conductor wire and making many turns or wraps to produce a winding. Converting the conductor from a single, isolated straight wire to one that contains many turns forming a winding amplifies the magnetic force many times. The amount of magnetic field amplification is based on the number of turns in the winding and the amount of current flowing through the conductor.

In this configuration, the magnetic flux is moving through air, which is a poor conductor of magnetic energy, thus allowing the magnetic flux to spread out over a very wide area. Therefore, the reluctance from the magnetic field when moving through air is quite high. Reluctance is a measure of how difficult it is for the magnetic flux to complete its circuit—that is, to leave one pole and enter the opposite pole. If the magnetic flux is kept close to the magnet, it has less resistance or opposition to flow.

Magnetic Principles and Motor Theory

All machine designs involving rotating equipment ultimately rely on theory to guide the engineer's application choices. Hence, a very brief review of magnetic principles and motor theory is always a convenient starting point for any discussion of DC motor applications. The laws of physics have blessed the world of machine design with the existence of magnetism, which is the foundation of motor theory. In essence, magnets, permanent or electromagnetic, produce fields of magnetic flux. These magnetic fields can produce an induced EMF through a coil of wire when relative movement between the field and a current carrying conductor occurs; and if this movement is reversed, so is the direction of the magnetic field, according to Faraday's Law. Thus, in theory, motor action or torque is produced when electrical energy is applied to conductor in a changing magnetic field, causing current flow in the conductor, generating both an induced EMF and a CEMF (Lenz's Law) resulting in rotational or mechanical energy..

DC Motors: Physical and Functional Descriptions

DC motors are commonly used in industrial machinery because of their inherent advantages—good speed control, high starting torque, reliable control methodology—which generally outweigh the increased maintenance costs associated with them.

Construction

The generic DC motor is constructed 13 with armature and field windings, interpoles, a frame or stator, a segmented commutator, a brush assembly and end bells. The rotating armature winding is wound on a laminated core, mounted on a steel shaft, supported by shaft bearings, and is connected to the segmented commutator that receives external

DC power through the brush assembly. Brushes conduct the current from external DC power circuit to the commutator and finally to the armature windings. The frame or stator supports the field windings and interpoles. The end bells encase all the parts of the motor into one unit.

Operation
DC motors produce torque and mechanical motion due to the interaction of the magnetic fields of the rotating armature coil and the stationary field coil mounted on the frame. The changing magnetic field of the armature is possible through the use of electrically conductive carbon brushes, which ride on the segmented, commutator ring; external DC power is applied to the brushes through the commutator to the armature windings. As current flows through the armature coil, a magnetic field results. The field windings mounted on the frame, also set up a magnetic field. After the rotating armature passes through half of a complete rotation, the commutator switches the direction of the current flow, thereby changing the direction of the magnetic field in the armature winding. This change produces opposing magnetic fields and sustains torque and rotation through the next half cycle of rotation until the commutator changes the direction of current flow and the magnetic field again.

Types
The field and armature windings of DC motors can be connected in series, shunt (parallel) or series-shunt to achieve different kinds of speed-torque characteristics. Hence, the three general categories of wound field DC motors are shunt-wound, series-wound and compound-wound. In series-wound motors, the armature is connected in series with the field to provide high starting torque; however, they do not operate at no-load: when speed decreases, torque increases, which can create a possibly unsafe runaway condition. In shunt wound motors, the armature and field are connected in parallel. This wiring arrangement produces an inverse speed-torque relationship: as speed increases, torque decreases. The compound-wound is a combination of a series- and shunt-wound motor by placing the field winding in series with the armature in addition to a shunt field. This type offers a combination of good starting torque and speed control.

Brushless motors are a hybrid type of DC motor that does not use a commutator. Rather, it is constructed with a permanent magnet rotor, optical shaft encoder that gives positional feedback information, a DC controller that excites the phase of stator windings required to develop torque based upon the encoder's feedback. Brushless motors characteristically have high maximum operating speeds, high torque to weight ratios and are compact in design (fractional horsepower). They are typically used in robotic arm applications.

Associated Solid State Controls
In order to supply the answer, it is necessary to examine some of the basic characteristics obtainable from DC motors and their associated solid state controls.
1. Wide speed range.
2. Good speed regulation.
3. Compact size and light weight (relative to mechanical variable speed).
4. Ease of control.
5. Low maintenance.
6. Low cost.

In order to realize how a DC drive has the capability to provide the above characteristics, the DC drive has to be analyzed as two elements that make up the package. These two elements are of course the motor and the control. (The "control" is more accurately called the "regulator"). Basic DC motors as used on nearly all packaged drives have a very simple performance characteristic the shaft turns at a speed almost directly proportional to the voltage applied to the armature.

External Adjustment
In addition to the normal external adjustment such as the speed potentiometer. there are a number of common internal adjustments that are used on simple small analog type SCR Drives (Silicon Controlled Rectifier Drive). Some of these adjustments are as follows:

- ✓ Minimum Speed
- ✓ Maximum Speed
- ✓ Current Limit (Torque Limit) . IR Compensation
- ✓ Acceleration Time . Deceleration Time

The following is a description of the function that these individual adjustments serve and their typical use.

Minimum Speed
In most cases when the control is initially installed the speed potentiometer can be turned down to its lowest point and the output voltage from the control will go to zero causing the motor to stop. There are many situations where this is not desirable. For example there are some machines that want to be kept running at a minimum speed and accelerated up to operating speed as necessary. There is also a possibility that an operator may use the speed potentiometer to stop the motor to work on the machine. This can be a dangerous situation since the motor has only been brought to a stop by zeroing the input signal voltage. A more desirable situation is when the motor is stopped by opening the circuit to the motor or power to the control using the on/off switch. By adjusting the minimum speed up to some point where the motor continues to run even with the speed potentiometer set to its lowest point, the operator must shut the control off to stop the motor. This adds a little safety into the system. The typical minimum speed adjustment is from 0 to 30% of motor base speed.

Maximum Speed
The maximum speed adjustment sets the maximum speed attainable either by raising the input signal to its maximum point or turning the potentiometer to the maximum point. For example on a typical DC motor the rated speed of the motor might 1750 RPM but the control might be capable of running it up to 1850 or 1900 RPM. In some cases it's desirable to limit the motor (and machine speed) to something less than would be available at this maximum setting. The maximum adjustment allows this to be done. By turning the internal potentiometer to a lower point the maximum output voltage from the control is limited. This limits the maximum speed available from the motor. In typical controls such as our BC140 the range of adjustment on the maximum speed is from 50 to 110% of motor base speed.

Current Limit
One very nice feature of electronic speed controls is that the current going to the motor is constantly monitored by the control. As mentioned previously, the current drawn by the armature of the DC motor is related to the torque that is required by the load. Since this monitoring and control is available an adjustment is provided in the control that limits the output current to a maximum value.

This function can be used to set a threshold point that will cause the motor to stall rather than putting out an excessive amount of torque. This capability gives the motor/control combination the ability to prevent damage that might otherwise occur if higher values of torque were available. This is handy on machines that might become jammed or otherwise stalled. It can also be used where the control is operating a device such as the center winder where the important thing becomes torque rather than the speed. In this case the current limit is set and the speed goes up or down to hold the tension 0 the material being wound. The current limit is normally factory set at 150% of the motor's rated current. This allows the motor to produce enough torque to start and accelerate the load and yet will not let the current (and torque) exceed 150% of its rated value when running. The range of adjustment is typically from 0 to 200% of the motor rated current.

IR Compensation
IR compensation is a method used to adjust for the droop in a motor's speed due to armature resistance. As mentioned previously, IR compensation is positive feedback that causes the control output voltage to rise slightly with increasing output current. This will help stabilize the motor's speed from a no load to full load condition. If the motor happens to be driving a load where the torque is constant or nearly so, then this adjustment is usually unnecessary. However, if the motor is driving a load with a widely fluctuating torque requirement, and speed regulation is critical, then IR compensation can be adjusted to stabilize the speed from the light load to full load condition. One caution is that when IR compensation is adjusted too high it results in an increasing speed characteristic. This means that as the load is applied the motor is actually going to be forced to run faster. When this happens it increases the voltage and current to the motor which in turn increases the motor speed further. If this adjustment is set too high an unstable "hunting" or oscillating condition occurs that is undesirable.

Acceleration Time Adjustment
The Acceleration Time adjustment performs the function that is indicated by its name. It will extend or shorten the amount of time for the motor to go from zero speed up to the set speed. It also regulates the time it takes to change speeds from one setting (say 50%) to another setting (perhaps 100%). So this setting has the ability to moderate the acceleration rate on the drive.

A couple notes are important: if an acceleration time that is too rapid is called for "acceleration time" will be overridden by the current limit. Acceleration will only occur at a rate that is allowed by the amount of current the control passes through to the motor. Also important to note is that on most small controls the acceleration time is not linear. What this means is that a change of 50 RPM may occur more rapidly when the motor is at low speed than it does when the motor is approaching the set point speed. This is important to know but usually not critical on simple applications where these drives are used.

Deceleration Time
This is an adjustment that allows loads to be slowed over an extended period of time. For example, if power is removed from the motor and the load stops in 3 seconds, then the decel time adjustment would allow you ”to increase that time and "power down" the load over a period of 4, 5, 6 or more seconds. Note: On a conventional simple DC drive it will not allow for the shortening of the time below the "coast to rest" time.

Adjustment Summary
The ability to adjust these six adjustments gives great flexibility to the typical inexpensive DC drive. In most cases the factory preset settings are adequate and need not be changed, but on other applications it may be desirable to tailor the characteristics of the control to the specific application. Many of these adjustments are available in other types of controls, such as variable frequency drives.

Understanding A-C Motors
AC Motor History
In 1882, Nikola Tesla discovered the rotating magnetic field, and pioneered the use of a rotary field of force to operate machines. He exploited the principle to design a unique two-phase induction motor in 1883. In 1885, Galileo Ferraris independently researched the concept. In 1888, Ferraris published his research in a paper to the Royal Academy of Sciences in Turin. Tesla had suggested that the commutators from a machine could be removed and the device could operate on a rotary field of force. Professor Poeschel, his teacher, stated that would be akin to building a perpetual motion machine.

Michail Osipovich Dolivo-Dobrovolsky later developed a three-phase "cage-rotor" in 1890. This type of motor is now used for the vast majority of commercial applications.

An AC motor has two parts: a stationary stator having coils supplied with alternating current to produce a rotating magnetic field, and a rotor attached to the output shaft that is given a torque by the rotating field.

AC Motor with Sliding Rotor
A conical-rotor brake motor incorporates the brake as an integral part of the conical sliding rotor. When the motor is at rest, a spring acts on the sliding rotor and forces the brake ring against the brake cap in the motor, holding the rotor stationary. When the motor is energized, its magnetic field generates both an axial and a radial component. The axial component overcomes the spring force, releasing the brake; while the radial component causes the rotor to turn. There is no additional brake control required.

Synchronous Electric Motor
A synchronous electric motor is an AC motor distinguished by a rotor spinning with coils passing magnets at the same rate as the alternating current and resulting magnetic field which drives it. Another way of saying this is that it has zero slip under usual operating conditions. Contrast this with an induction motor, which must slip to produce torque. One type of synchronous motor is like an induction motor except the rotor is excited by a DC field. Slip rings and brushes are used to conduct current to the rotor. The rotor poles connect to each other and move at the same speed hence the name synchronous motor. Another type, for low load torque, has flats ground onto a conventional squirrel-cage rotor to create discrete poles. Yet another, such as made by Hammond for its pre-World War II clocks, and in the older Hammond organs, has no rotor windings and

discrete poles. It is not self-starting. The clock requires manual starting by a small knob on the back, while the older Hammond organs had an auxiliary starting motor connected by a spring-loaded manually operated switch.

Finally, hysteresis synchronous motors typically are (essentially) two-phase motors with a phase-shifting capacitor for one phase. They start like induction motors, but when slip rate decreases sufficiently, the rotor (a smooth cylinder) becomes temporarily magnetized. Its distributed poles make it act like a permanent-magnet-rotor synchronous motor. The rotor material, like that of a common nail, will stay magnetized, but can also be demagnetized with little difficulty. Once running, the rotor poles stay in place; they do not drift.

Low-power synchronous timing motors (such as those for traditional electric clocks) may have multi-pole permanent-magnet external cup rotors, and use shading coils to provide starting torque. Telechron clock motors have shaded poles for starting torque, and a two-spoke ring rotor that performs like a discrete two-pole rotor.

Induction Motor

An induction motor is an asynchronous AC motor where power is transferred to the rotor by electromagnetic induction, much like transformer action. An induction motor resembles a rotating transformer, because the stator (stationary part) is essentially the primary side of the transformer and the rotor (rotating part) is the secondary side. Polyphase induction motors are widely used in industry.

Induction motors may be further divided into squirrel-cage motors and wound-rotor motors. Squirrel-cage motors have a heavy winding made up of solid bars, usually aluminum or copper, joined by rings at the ends of the rotor. When one considers only the bars and rings as a whole, they are much like an animal's rotating exercise cage, hence the name.

Currents induced into this winding provide the rotor magnetic field. The shape of the rotor bars determines the speed-torque characteristics. At low speeds, the current induced in the squirrel cage is nearly at line frequency and tends to be in the outer parts of the rotor cage. As the motor accelerates, the slip frequency becomes lower, and more current is in the interior of the winding. By shaping the bars to change the resistance of the winding portions in the interior and outer parts of the cage, effectively a variable resistance is inserted in the rotor circuit. However, the majority of such motors have uniform bars.

In a wound-rotor motor, the rotor winding is made of many turns of insulated wire and is connected to slip rings on the motor shaft. An external resistor or other control devices can be connected in the rotor circuit. Resistors allow control of the motor speed, although significant power is dissipated in the external resistance. A converter can be fed from the rotor circuit and return the slip-frequency power that would otherwise be wasted back into the power system through an inverter or separate motor-generator.

The wound-rotor induction motor is used primarily to start a high inertia load or a load that requires a very high starting torque across the full speed range. By correctly selecting the resistors used in the secondary resistance or slip ring starter, the motor is able to produce maximum torque at a relatively low supply current from zero speed to full speed. This type of motor also offers controllable speed.

Motor speed can be changed because the torque curve of the motor is effectively modified by the amount of resistance connected to the rotor circuit. Increasing the value of resistance will move the speed of maximum torque down. If the resistance connected to the rotor is increased beyond the point where the maximum torque occurs at zero speed, the torque will be further reduced.

When used with a load that has a torque curve that increases with speed, the motor will operate at the speed where the torque developed by the motor is equal to the load torque. Reducing the load will cause the motor to speed up, and increasing the load will cause the motor to slow down until the load and motor torque are equal. Operated in this manner, the slip losses are dissipated in the secondary resistors and can be very significant. The speed regulation and net efficiency is also very poor. Various regulatory authorities in many countries have introduced and implemented legislation to encourage the manufacture and use of higher efficiency electric motors.

Doubly Fed Electric Motor
Doubly fed electric motors have two independent multiphase winding sets, which contribute active (i.e., working) power to the energy conversion process, with at least one of the winding sets electronically controlled for variable speed operation. Two independent multiphase winding sets (i.e., dual armature) are the maximum provided in a single package without topology duplication. Doubly fed electric motors are machines with an effective constant torque speed range that is twice synchronous speed for a given frequency of excitation. This is twice the constant torque speed range as singly fed electric machines, which have only one active winding set.

A doubly fed motor allows for a smaller electronic converter but the cost of the rotor winding and slip rings may offset the saving in the power electronics components. Difficulties with controlling speed near synchronous speed limit applications.

Singly Fed Electric Motor
Most AC motors are singly fed. Singly fed electric motors have a single multiphase winding set that is connected to a power supply. Singly fed electric machines may be either induction or synchronous. The active winding set can be electronically controlled. Singly fed electric machines have an effective constant torque speed range up to synchronous speed for a given excitation frequency.

Torque Motors
A torque motor (also known as a limited torque motor) is a specialized form of induction motor which is capable of operating indefinitely while stalled, that is, with the rotor blocked from turning, without incurring damage. In this mode of operation, the motor will apply a steady torque to the load (hence the name).

A common application of a torque motor would be the supply- and take-up reel motors in a tape drive. In this application, driven from a low voltage, the characteristics of these motors allow a relatively constant light tension to be applied to the tape whether or not the capstan is feeding tape past the tape heads. Driven from a higher voltage, (and so delivering a higher torque), the torque motors can also achieve fast-forward and rewind operation without requiring any additional mechanics such as gears or clutches. In the computer gaming world, torque motors are used in force feedback steering wheels.

Another common application is the control of the throttle of an internal combustion engine in conjunction with an electronic governor. In this usage, the motor works against a return spring to move the throttle in accordance with the output of the governor. The latter monitors engine speed by counting electrical pulses from the ignition system or from a magnetic pickup[27] and, depending on the speed, makes small adjustments to the amount of current applied to the motor. If the engine starts to slow down relative to the desired speed, the current will be increased, the motor will develop more torque, pulling against the return spring and opening the throttle. Should the engine run too fast, the governor will reduce the current being applied to the motor, causing the return spring to pull back and close the throttle.

Stepper Motors

Closely related in design to three-phase AC synchronous motors are stepper motors, where an internal rotor containing permanent magnets or a magnetically soft rotor with salient poles is controlled by a set of external magnets that are switched electronically. A stepper motor may also be thought of as a cross between a DC electric motor and a rotary solenoid. As each coil is energized in turn, the rotor aligns itself with the magnetic field produced by the energized field winding. Unlike a synchronous motor, in its application, the stepper motor may not rotate continuously; instead, it "steps"—starts and then quickly stops again—from one position to the next as field windings are energized and de-energized in sequence. Depending on the sequence, the rotor may turn forwards or backwards, and it may change direction, stop, speed up or slow down arbitrarily at any time.

Simple stepper motor drivers entirely energize or entirely de-energize the field windings, leading the rotor to "cog" to a limited number of positions; more sophisticated drivers can proportionally control the power to the field windings, allowing the rotors to position between the cog points and thereby rotate extremely smoothly. This mode of operation is often called microstepping. Computer controlled stepper motors are one of the most versatile forms of positioning systems, particularly when part of a digital servo-controlled system.

Stepper motors can be rotated to a specific angle in discrete steps with ease, and hence stepper motors are used for read/write head positioning in computer floppy diskette drives. They were used for the same purpose in pre-gigabyte era computer disk drives, where the precision and speed they offered was adequate for the correct positioning of the read/write head of a hard disk drive. As drive density increased, the precision and speed limitations of stepper motors made them obsolete for hard drives—the precision limitation made them unusable, and the speed limitation made them uncompetitive—thus newer hard disk drives use voice coil-based head actuator systems. (The term "voice coil" in this connection is historic; it refers to the structure in a typical (cone type) loudspeaker. This structure was used for a while to position the heads. Modern drives have a pivoted coil mount; the coil swings back and forth, something like a blade of a rotating fan. Nevertheless, like a voice coil, modern actuator coil conductors (the magnet wire) move perpendicular to the magnetic lines of force.)

Stepper motors were and still are often used in computer printers, optical scanners, and digital photocopiers to move the optical scanning element, the print head carriage (of dot matrix and inkjet printers), and the platen or feed rollers. Likewise, many computer plotters (which since the early 1990s have been replaced with large-format inkjet and laser printers) used rotary stepper motors for pen and platen movement; the typical

alternatives here were either linear stepper motors or servomotors with closed-loop analog control systems.

So-called quartz analog wristwatches contain the smallest commonplace stepping motors; they have one coil, draw very little power, and have a permanent-magnet rotor. The same kind of motor drives battery-powered quartz clocks. Some of these watches, such as chronographs, contain more than one stepping motor.

Rotary
Uses include rotating machines such as fans, turbines, drills, the wheels on electric cars, locomotives and conveyor belts. Also, in many vibrating or oscillating machines, an electric motor spins an unbalanced mass, causing the motor (and its mounting structure) to vibrate. A familiar application is cell phone vibrating alerts used when the acoustic "ringer" is disabled by the user.

Electric motors are also popular in robotics. They turn the wheels of vehicular robots, and servo motors operate arms in industrial robots; they also move arms and legs in humanoid robots. In flying robots, along with helicopters, a motor rotates a propeller, or aerodynamic rotor blades to create controllable amounts of lift. Electric motors are replacing hydraulic cylinders in airplanes and military equipment.

In industrial and manufacturing businesses, electric motors rotate saws and blades in cutting and slicing processes; they rotate parts being turned in lathes and other machine tools, and spin grinding wheels. Fast, precise servo motors position tools and work in modern CNC machine tools. Motor-driven mixers are very common in food manufacturing. Linear motors are often used to push products into containers horizontally.

Many kitchen appliances also use electric motors. Food processors and grinders spin blades to chop and break up foods. Blenders use electric motors to mix liquids, and microwave ovens use motors to turn the tray that food sits on. Toaster ovens also use electric motors to turn a conveyor to move food over heating elements.

Servo Motor
A servomotor is a motor, very often sold as a complete module, which is used within a position-control or speed-control feedback control system mainly control valves, such as motor operated control valves. Servomotors are used in applications such as machine tools, pen plotters, and other process systems. Motors intended for use in a servomechanism must have well-documented characteristics for speed, torque, and power. The speed vs. torque curve is quite important and is high ratio for a servo motor. Dynamic response characteristics such as winding inductance and rotor inertia are also important; these factors limit the overall performance of the servomechanism loop. Large, powerful, but slow-responding servo loops may use conventional AC or DC motors and drive systems with position or speed feedback on the motor. As dynamic response requirements increase, more specialized motor designs such as coreless motors are used.

A servo system differs from some stepper motor applications in that the position feedback is continuous while the motor is running; a stepper system relies on the motor not to "miss steps" for short term accuracy, although a stepper system may include a "home" switch or other element to provide long-term stability of control. For instance,

when a typical dot matrix computer printer starts up, its controller makes the print head stepper motor drive to its left-hand limit, where a position sensor defines home position and stops stepping. As long as power is on, a bidirectional counter in the printer's microprocessor keeps track of print-head position.

Linear Motor

A linear motor is essentially any electric motor that has been "unrolled" so that, instead of producing a torque (rotation), it produces a straight-line force along its length. Linear motors are most commonly induction motors or stepper motors. Linear motors are commonly found in many roller-coasters where the rapid motion of the motorless railcar is controlled by the rail. They are also used in maglev trains, where the train "flies" over the ground. On a smaller scale, the HP 7225A pen plotter, released in 1978, used two linear stepper motors to move the pen along the X and Y axes.

Torque Capability of Motor Types

When optimally designed within a given core saturation constraint and for a given active current (i.e., torque current), voltage, pole-pair number, excitation frequency (i.e., synchronous speed), and air-gap flux density, all categories of electric motors or generators will exhibit virtually the same maximum continuous shaft torque (i.e., operating torque) within a given air-gap area with winding slots and back-iron depth, which determines the physical size of electromagnetic core. Some applications require bursts of torque beyond the maximum operating torque, such as short bursts of torque to accelerate an electric vehicle from standstill. Always limited by magnetic core saturation or safe operating temperature rise and voltage, the capacity for torque bursts beyond the maximum operating torque differs significantly between categories of electric motors or generators.

Capacity for bursts of torque should not be confused with field weakening capability inherent in fully electromagnetic electric machines (Permanent Magnet (PM) electric machine are excluded). Field weakening, which is not available with PM electric machines, allows an electric machine to operate beyond the designed frequency of excitation.

Electric machines without a transformer circuit topology, such as Field-Wound (i.e., electromagnet) or Permanent Magnet (PM) Synchronous electric machines cannot realize bursts of torque higher than the maximum designed torque without saturating the magnetic core and rendering any increase in current as useless. Furthermore, the permanent magnet assembly of PM synchronous electric machines can be irreparably damaged, if bursts of torque exceeding the maximum operating torque rating are attempted.

Electric machines with a transformer circuit topology, such as Induction (i.e., asynchronous) electric machines, Induction Doubly Fed electric machines, and Induction or Synchronous Wound-Rotor Doubly Fed (WRDF) electric machines, exhibit very high bursts of torque because the active current (i.e., Magneto-Motive-Force or the product of current and winding-turns) induced on either side of the transformer oppose each other and as a result, the active current contributes nothing to the transformer coupled magnetic core flux density, which would otherwise lead to core saturation.

Electric machines that rely on Induction or Asynchronous principles short-circuit one port of the transformer circuit and as a result, the reactive impedance of the transformer circuit becomes dominant as slip increases, which limits the magnitude of active (i.e.,

real) current. Still, bursts of torque that are two to three times higher than the maximum design torque are realizable.

The Synchronous WRDF electric machine is the only electric machine with a truly dual ported transformer circuit topology (i.e., both ports independently excited with no short-circuited port). The dual ported transformer circuit topology is known to be unstable and requires a multiphase slip-ring-brush assembly to propagate limited power to the rotor winding set. If a precision means were available to instantaneously control torque angle and slip for synchronous operation during motoring or generating while simultaneously providing brushless power to the rotor winding set (see Brushless wound-rotor doubly fed electric machine), the active current of the Synchronous WRDF electric machine would be independent of the reactive impedance of the transformer circuit and bursts of torque significantly higher than the maximum operating torque and far beyond the practical capability of any other type of electric machine would be realizable. Torque bursts greater than eight times operating torque have been calculated.

Continuous Torque Density
The continuous torque density of conventional electric machines is determined by the size of the air-gap area and the back-iron depth, which are determined by the power rating of the armature winding set, the speed of the machine, and the achievable air-gap flux density before core saturation. Despite the high coercivity of neodymium or samarium-cobalt permanent magnets, continuous torque density is virtually the same amongst electric machines with optimally designed armature winding sets. Continuous torque density should never be confused with peak torque density, which comes with the manufacturer's chosen method of cooling, which is available to all, or period of operation before destruction by overheating of windings or even permanent magnet damage.

Continuous Power Density
The continuous power density is determined by the product of the continuous torque density and the constant torque speed range of the electric machine.

Understanding Single Phase
In electrical engineering, single-phase electric power refers to the distribution of alternating current electric power using a system in which all the voltages of the supply vary in unison. Single-phase distribution is used when loads are mostly lighting and heating, with few large electric motors. A single-phase supply connected to an alternating current electric motor does not produce a revolving magnetic field; single-phase motors need additional circuits for starting, and such motors are uncommon above 10 or 20 kW in rating.

In contrast, in a three-phase system, the currents in each conductor reach their peak instantaneous values sequentially, not simultaneously; in each cycle of the power frequency, first one, then the second, then the third current reaches its maximum value. The waveforms of the three supply conductors are offset from one another in time (delayed in phase) by one-third of their period. When the three phases are connected to windings around the interior of a motor stator, they produce a revolving magnetic field; such motors are self-starting.

Standard Frequencies of Single-Phase Power
Standard frequencies of single-phase power systems are either 50 or 60 Hz. Special single-phase traction power networks may operate at 16.67 Hz or other frequencies to

power electric railways. In some countries such as the United States, single phase is commonly divided in half to create split-phase electric power for household appliances and lighting.

Single-phase power distribution is widely used especially in rural areas, where the cost of a three-phase distribution network is high and motor loads are small and uncommon.

High power systems, say, hundreds of kVA or larger, are nearly always three phase. The largest supply normally available as single phase varies according to the standards of the electrical utility. In the UK a single-phase household supply may be rated 100 A or even 125 A, meaning that there is little need for 3 phase in a domestic or small commercial environment. Much of the rest of Europe has traditionally had much smaller limits on the size of single phase supplies resulting in even houses being supplied with 3 phase (in urban areas with three-phase supply networks).

In North America, individual residences and small commercial buildings with services up to about 100 kV·A (417 amperes at 240 volts) will usually have three-wire single-phase distribution, often with only one customer per distribution transformer. In exceptional cases larger single-phase three-wire services can be provided, usually only in remote areas where poly-phase distribution is not available. In rural areas farmers who wish to use three-phase motors may install a phase converter if only a single-phase supply is available. Larger consumers such as large buildings, shopping centers, factories, office blocks, and multiple-unit apartment blocks will have three-phase service. In densely populated areas of cities, network power distribution is used with many customers and many supply transformers connected to provide hundreds or thousands of kV·A, a load concentrated over a few hundred square meters.

Understanding Three Phase

Three-phase electric power is a common method of alternating-current electric power generation, transmission, and distribution.[1] It is a type of polyphase system and is the most common method used by electrical grids worldwide to transfer power. It is also used to power large motors and other heavy loads. A three-phase system is generally more economical than others because it uses less conductor material to transmit electric power than equivalent single-phase or two-phase systems at the same voltage. The three-phase system was introduced and patented by Nikola Tesla in 1887 and 1888.

In a three-phase system, three circuit conductors carry three alternating currents (of the same frequency) which reach their instantaneous peak values at different times. Taking one conductor as the reference, the other two currents are delayed in time by one-third and two-thirds of one cycle of the electric current. This delay between phases has the effect of giving constant power transfer over each cycle of the current and also makes it possible to produce a rotating magnetic field in an electric motor.

Three-phase systems may have a neutral wire. A neutral wire allows the three-phase system to use a higher voltage while still supporting lower-voltage single-phase appliances. In high-voltage distribution situations, it is common not to have a neutral wire as the loads can simply be connected between phases (phase-phase connection).

Three-phase has properties that make it very desirable in electric power systems:

- ✓ The phase currents tend to cancel out one another, summing to zero in the case of a linear balanced load. This makes it possible to eliminate or reduce the size

of the neutral conductor; all the phase conductors carry the same current and so can be the same size, for a balanced load.

- ✓ Power transfer into a linear balanced load is constant, which helps to reduce generator and motor vibrations.
- ✓ Three-phase systems can produce a magnetic field that rotates in a specified direction, which simplifies the design of electric motors.
- ✓ Three is the lowest phase order to exhibit all of these properties.

Most household loads are single-phase. In North America and a few other places, three-phase power generally does not enter homes. Even in areas where it does, it is typically split out at the main distribution board and the individual loads are fed from a single phase. Sometimes it is used to power electric stoves and electric clothes dryers.

3 Or 4 Wire

Three-phase circuits occur in two varieties: three-wire and four-wire. Both types have three energized ("hot" or "live") wires, but the 4-wire circuit also has neutral wire. The three-wire system is used when the loads on the 3 live wires will be balanced, for example in motors or heating elements with 3 identical coils. The neutral wire is used when there is a chance that the loads are not balanced. A common example of this is local distribution in Europe, where each house will be connected to just one of the live wires, but all connected to the same neutral. The neutral carries the "imbalance" between the power carried on the 3 live wires. Hence electrical engineers work hard to make sure that the power is shared around equally, so the neutral wire carries as little power as possible and can therefore be made much smaller than the other 3. The '3-wire' and '4-wire' designations do not count the ground wire used on many transmission lines, as this is solely for fault and lightning protection and does not serve to deliver electrical power.

The most important class of three-phase load is the electric motor. A three-phase induction motor has a simple design, inherently high starting torque and high efficiency. Such motors are applied in industry for pumps, fans, blowers, compressors, conveyor drives, electric vehicles and many other kinds of motor-driven equipment. A three-phase motor is more compact and less costly than a single-phase motor of the same voltage class and rating and single-phase AC motors above 10 HP (7.5 kW) are uncommon. Three-phase motors also vibrate less and hence last longer than single-phase motors of the same power used under the same conditions.

Resistance heating loads such as electric boilers or space heating may be connected to three-phase systems. Electric lighting may also be similarly connected. These types of loads do not require the revolving magnetic field characteristic of three-phase motors but take advantage of the higher voltage and power level usually associated with three-phase distribution. Legacy single-phase fluorescent lighting systems also benefit from reduced flicker in a room if adjacent fixtures are powered from different phases.

Large rectifier systems may have three-phase inputs; the resulting DC is easier to filter (smooth) than the output of a single-phase rectifier. Such rectifiers may be used for battery charging, electrolysis processes such as aluminum production or for operation of DC motors.

Phase Converters
Occasionally the advantages of three-phase motors make it worthwhile to convert single-phase power to three-phase. Small customers, such as residential or farm properties, may not have access to a three-phase supply or may not want to pay for the extra cost of a three-phase service but may still wish to use three-phase equipment. Such converters may also allow the frequency to be varied (resynthesis) allowing speed control. Some railway locomotives are moving to multi-phase motors driven by such systems even though the incoming supply to a locomotive is nearly always either DC or single-phase AC.

Because single-phase power goes to zero at each moment that the voltage crosses zero but three-phase delivers power continuously, any such converter must have a way to store the necessary energy for a fraction of a second.

One method for using three-phase equipment on a single-phase supply is with a rotary phase converter, essentially a three-phase motor with special starting arrangements and power factor correction that produces balanced three-phase voltages. When properly designed, these rotary converters can allow satisfactory operation of three-phase equipment such as machine tools on a single-phase supply. In such a device, the energy storage is performed by the mechanical inertia (flywheel effect) of the rotating components. An external flywheel is sometimes found on one or both ends of the shaft.

A second method that was popular in the 1940s and 1950s was the transformer method. At that time, capacitors were more expensive than transformers, so an autotransformer was used to apply more power through fewer capacitors. This method performs well and does have supporters, even today. The usage of the name transformer method separated it from another common method, the static converter, as both methods have no moving parts, which separates them from the rotary converters.

Another method often attempted is with a device referred to as a static phase converter. This method of running three-phase equipment is commonly attempted with motor loads though it only supplies power and can cause the motor loads to run hot and in some cases overheat. This method does not work when sensitive circuitry is involved such as CNC devices or in induction and rectifier-type loads.

A three-phase generator can be driven by a single-phase motor. This motor-generator combination can provide a frequency changer function as well as phase conversion, but requires two machines with all their expense and losses. The motor-generator method can also form an uninterruptable power supply when used in conjunction with a large flywheel and a standby generator set.

Some devices are made which create an imitation three-phase from three-wire single-phase supplies. This is done by creating a third "subphase" between the two live conductors, resulting in a phase separation of 180° - 90° = 90°. Many three-phase devices can run on this configuration but at lower efficiency.

Variable-frequency drives (also known as solid-state inverters) are used to provide precise speed and torque control of three-phase motors. Some models can be powered by a single-phase supply. VFDs work by converting the supply voltage to DC and then converting the DC to a suitable three-phase source for the motor.

Digital phase converters are designed for fixed-frequency operation from a single-phase source. Similar to a variable-frequency drive, they use a microprocessor to control solid-state power switching components to maintain balanced three-phase voltages.

Alternatives to Three-Phase

- ✓ Split-phase electric power is used when three-phase power is not available and allows double the normal utilization voltage to be supplied for high-power loads.

- ✓ Two-phase electric power, like three-phase, gives constant power transfer to a linear load. For loads that connect each phase to neutral, assuming the load is the same power draw, the two-wire system has a neutral current which is greater than neutral current in a three-phase system. Also motors are not entirely linear, which means that despite the theory, motors running on three-phase tend to run smoother than those on two-phase. The generators in the Adams Power Plant at Niagara Falls which were installed in 1895 were the largest generators in the world at the time and were two-phase machines. True two-phase power distribution is basically obsolete. Special-purpose systems may use a two-phase system for control. Two-phase power may be obtained from a three-phase system (or vice versa) using an arrangement of transformers called a Scott-T transformer.

- ✓ Monocyclic power was a name for an asymmetrical modified two-phase power system used by General Electric around 1897, championed by Charles Proteus Steinmetz and Elihu Thomson. This system was devised to avoid patent infringement. In this system, a generator was wound with a full-voltage single-phase winding intended for lighting loads and with a small fraction (usually ¼ of the line voltage) winding which produced a voltage in quadrature with the main windings. The intention was to use this "power wire" additional winding to provide starting torque for induction motors, with the main winding providing power for lighting loads. After the expiration of the Westinghouse patents on symmetrical two-phase and three-phase power distribution systems, the monocyclic system fell out of use; it was difficult to analyze and did not last long enough for satisfactory energy metering to be developed.

- ✓ High-phase-order systems for power transmission have been built and tested. Such transmission lines use six (two-pole, three-phase) or twelve (two-pole, six-phase) lines and employ design practices characteristic of extra-high-voltage transmission lines. High-phase-order transmission lines may allow transfer of more power through a given transmission line right-of-way without the expense of a high-voltage direct current (HVDC) converter at each end of the line.

Understanding Slip Ring Motors

A slip ring (in electrical engineering terms) is a method of making an electrical connection through a rotating assembly. Slip rings, also called rotary electrical interfaces, rotating electrical connectors, collectors, swivels, or electrical rotary joints,

are commonly found in slip ring motors, electrical generators for alternating current (AC) systems and alternators and in packaging machinery, cable reels, and wind turbines. They can be used on any rotating object to transfer power, control circuits, or analog or digital signals including data such as those found on aerodrome beacons, rotating tanks, power shovels, radio telescopes or heliostats.

A slip ring is a rotary coupling used to transfer electric current from a stationary unit to a rotating unit. Either the brushes or the rings are stationary and the other component rotates. This system is similar to the brushes and commutator, found in many types of DC motors. While commutators are segmented, slip rings are continuous, and the terms are not interchangeable. Rotary transformers are often used instead of slip rings in high speed or low friction environments.

The slip ring induction motors usually have “Phase-Wound” rotor. This type of rotor is provided with a 3-phase, double-layer, distributed winding consisting of coils used in alternators. The rotor core is made up of steel laminations which has slots to accommodate formed 3-single phase windings. These windings are placed 120 degrees electrically apart.

The rotor is wound for as many poles as the number of poles in the stator and is always 3-phase, even though the stator is wound for 2-phase. These three windings are “starred” internally and other end of these three windings are brought out and connected to three insulated slip-rings mounted on the rotor shaft itself. The three terminal ends touch these three slip rings with the help of carbon brushes which are held against the rings with the help of spring assembly.

These three carbon brushes are further connected externally to a 3-phase start connected rheostat. Thus these slip ring and external rheostat makes the slip ring induction motors possible to add external resistance to the rotor circuit, thus enabling them to have a higher resistance during starting and thus higher starting torque.

When running during normal condition, the slip rings are automatically short-circuited by means of a metal collar, which is pushed along the shaft, thus making the three rings touching each other. Also, the brushes are automatically lifted from the slip-rings to avoid frictional losses, wear and tear. Hence, under normal running conditions, the wound rotor is acting as same as the squirrel cage rotor.

Mercury-wetted slip rings, noted for their low resistance and stable connection use a different principle which replaces the sliding brush contact with a pool of liquid metal molecularly bonded to the contacts. During rotation the liquid metal maintains the electrical connection between the stationary and rotating contacts. However, the use of mercury poses safety concerns, as it is a toxic substance. If a slip ring application involves food manufacturing or processing, pharmaceutical equipment, or any other use where contamination could be a serious threat, the choice should be precious metal contacts. Leakage of the mercury and the resultant contamination could be extremely serious. The slip ring device is also limited by temperature, as mercury solidifies at approximately -40 C.

A pancake slip ring has the conductors arranged on a flat disk as concentric rings centered on the rotating shaft. This configuration has greater weight and volume for the same circuits, greater capacitance and crosstalk, greater brush wear and more readily

collects wear debris on its vertical axis. However, a pancake offers reduced axial length for the number of circuits, and so may be appropriate in some applications. Slip rings are made in various sizes; one device made for theatrical stage lighting had 100 conductors. The slip ring allows for unlimited rotations of the connected object, whereas a slack cable can only be twisted a few times before it will fail.

Stator
The stator construction is same for both squirrel cage and slip ring induction motor. The main difference in slip ring induction motor is on the rotor construction and usage. Some changes in the stator may be encountered when a slip ring motor is used in a cascaded system, as the supply for the slave motor is controlled by the supply from rotor of other slip ring motor with external resistance mounted on its rotor.

A wound-rotor motor is a type of induction motor where the rotor windings are connected through slip rings to external resistances. Adjusting the resistance allows control of the speed/torque characteristic of the motor. Wound-rotor motors can be started with low inrush current, by inserting high resistance into the rotor circuit; as the motor accelerates, the resistance can be decreased.

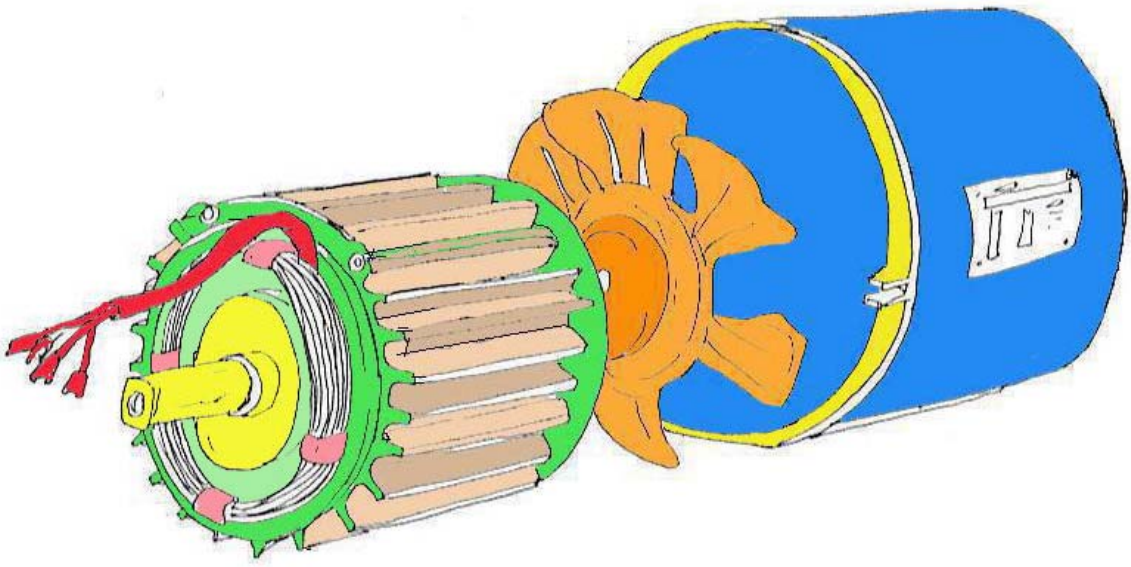
Compared to a squirrel-cage rotor, the rotor of the slip ring motor has more winding turns; the induced voltage is then higher, and the current lower, than for a squirrel-cage rotor. During the start-up a typical rotor has 3 poles connected to the slip ring. Each pole is wired in series with a variable power resistor. When the motor reaches full speed the rotor poles are switched to short circuit. During start-up the resistors reduce the field strength in the stator. As a result the inrush current is reduced. Another important advantage over squirrel-cage motors is higher start-up torque.

A wound-rotor motor can be used in several forms of adjustable-speed drive. Certain types of variable-speed drives recover slip-frequency power from the rotor circuit and feed it back to the supply, allowing wide speed range with high energy efficiency. Doubly fed electric machines use the slip rings to supply external power to the rotor circuit, allowing wide-range speed control. Today speed control by use of slip ring motor is mostly superseded by induction motors with variable-frequency drives.

Pump Repairs
Examining pump repair records and MTBF (mean time between failures) is of great importance to responsible and conscientious pump users. In view of that fact, the preface to the 2006 Pump User’s Handbook alludes to "pump failure" statistics. For the sake of convenience, these failure statistics often are translated into MTBF (in this case, installed life before failure).

Unscheduled maintenance is often one of the most significant costs of ownership, and failures of mechanical seals and bearings are among the major causes. Keep in mind the potential value of selecting pumps that cost more initially, but last much longer between repairs. The MTBF of a better pump may be one to four years longer than that of its non-upgraded counterpart. Consider that published average values of avoided pump failures range from $2600 to $12,000. This does not include lost opportunity costs. One pump fire occurs per 1000 failures. Having fewer pump failures means having fewer destructive pump fires.

More Detailed Information on Motors



The classic division of electric motors has been that of Direct Current (DC) types vs. Alternating Current (AC) types. This is more a de facto convention, rather than a rigid distinction. For example, many classic DC motors run happily on AC power.

The ongoing trend toward electronic control further muddles the distinction, as modern drivers have moved the commutator out of the motor shell. For this new breed of motor, driver circuits are relied upon to generate sinusoidal AC drive currents, or some approximation of. The two best examples are: the brushless DC motor and the stepping motor, both being polyphase AC motors requiring external electronic control.

There is a clearer distinction between a synchronous motor and asynchronous types. In the synchronous types, the rotor rotates in synchrony with the oscillating field or current (e.g. permanent magnet motors). In contrast, an asynchronous motor is designed to slip; the most ubiquitous example being the common AC induction motor which must slip in order to generate torque. A DC motor is designed to run on DC electric power. Two examples of pure DC designs are Michael Faraday's homopolar motor (which is uncommon), and the ball bearing motor, which is (so far) a novelty. By far the most common DC motor types are the brushed and brushless types, which use internal and external commutation respectively to create an oscillating AC current from the DC source -- so they are not purely DC machines in a strict sense.

Brushed DC Motors
The classic DC motor design generates an oscillating current in a wound rotor with a split ring commutator, and either a wound or permanent magnet stator. A rotor consists of a coil wound around a rotor, which is then powered by any type of battery. Many of the limitations of the classic commutator DC motor are due to the need for brushes to press against the commutator. This creates friction. At higher speeds, brushes have increasing difficulty in maintaining contact. Brushes may bounce off the irregularities in the commutator surface, creating sparks. This limits the maximum speed of the machine. The current density per unit area of the brushes limits the output of the motor.

The imperfect electric contact also causes electrical noise. Brushes eventually wear out and require replacement, and the commutator itself is subject to wear and maintenance. The commutator assembly on a large machine is a costly element, requiring precision assembly of many parts.

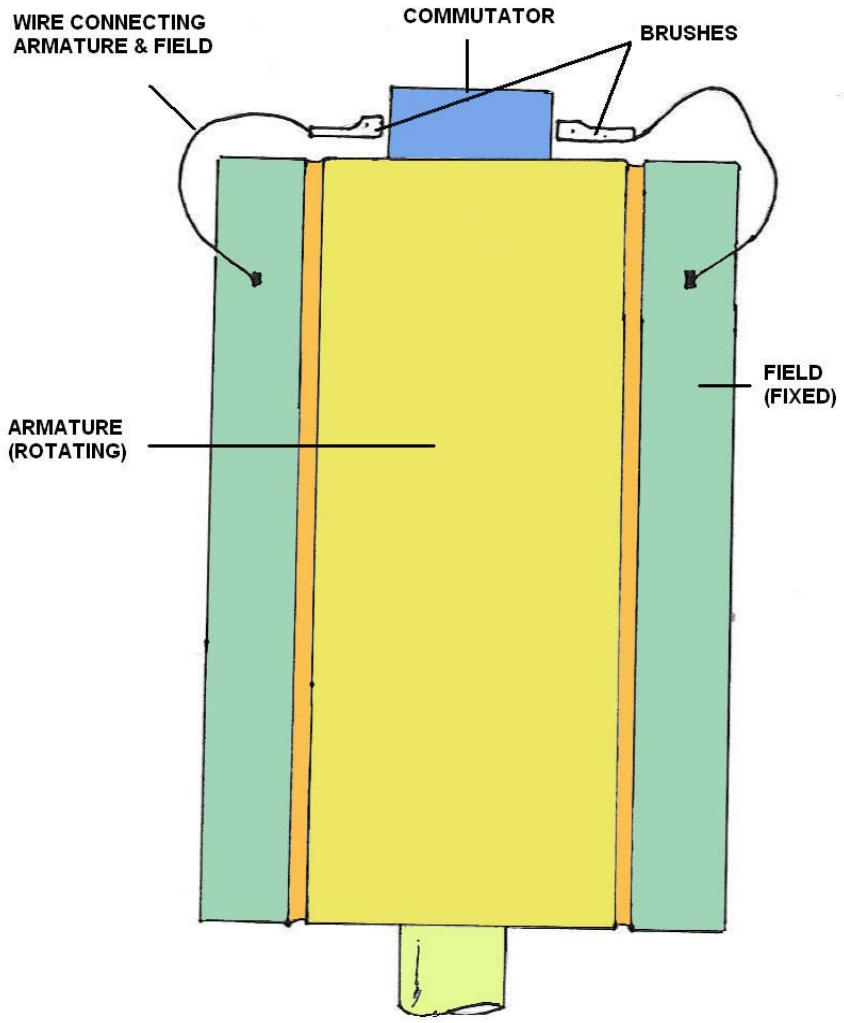


DIAGRAM SHOWING MECHANICAL CONSTRUCTION OF A DC SERIES WOUND MOTOR

Brushless DC Motors

Some of the problems of the brushed DC motor are eliminated in the brushless design. In this motor, the mechanical "rotating switch" or commutator/brush gear assembly is replaced by an external electronic switch synchronized to the rotor's position. Brushless motors are typically 85-90% efficient, whereas DC motors with brush gear are typically 75-80% efficient.

Midway between ordinary DC motors and stepper motors lies the realm of the brushless DC motor. Built in a fashion very similar to stepper motors, these often use a permanent magnet external rotor, three phases of driving coils, one or more Hall effect sensors to sense the position of the rotor, and the associated drive electronics.

The coils are activated one phase after the other by the drive electronics, as cued by the signals from the Hall effect sensors. In effect, they act as three-phase synchronous motors containing their own variable-frequency drive electronics. A specialized class of brushless DC motor controllers utilize EMF feedback through the main phase connections instead of Hall effect sensors to determine position and velocity.

Brushless DC motors are commonly used where precise speed control is necessary, as in computer disk drives or in video cassette recorders, the spindles within CD, CD-ROM (etc.) drives, and mechanisms within office products such as fans, laser printers, and photocopiers.

They have several advantages over conventional motors:
* Compared to AC fans using shaded-pole motors, they are very efficient, running much cooler than the equivalent AC motors. This cool operation leads to much-improved life of the fan's bearings.
* Without a commutator to wear out, the life of a DC brushless motor can be significantly longer compared to a DC motor using brushes and a commutator. Commutation also tends to cause a great deal of electrical and RF noise; without a commutator or brushes, a brushless motor may be used in electrically sensitive devices like audio equipment or computers.
* The same Hall effect sensors that provide the commutation can also provide a convenient tachometer signal for closed-loop control (servo-controlled) applications. In fans, the tachometer signal can be used to derive a "fan OK" signal.
* The motor can be easily synchronized to an internal or external clock, leading to precise speed control.
* Brushless motors have no chance of sparking, unlike brushed motors, making them better suited to environments with volatile chemicals and fuels.
* Brushless motors are usually used in small equipment such as computers and are generally used to get rid of unwanted heat.
* They are also very quiet motors, which is an advantage if being used in equipment that is affected by vibrations.

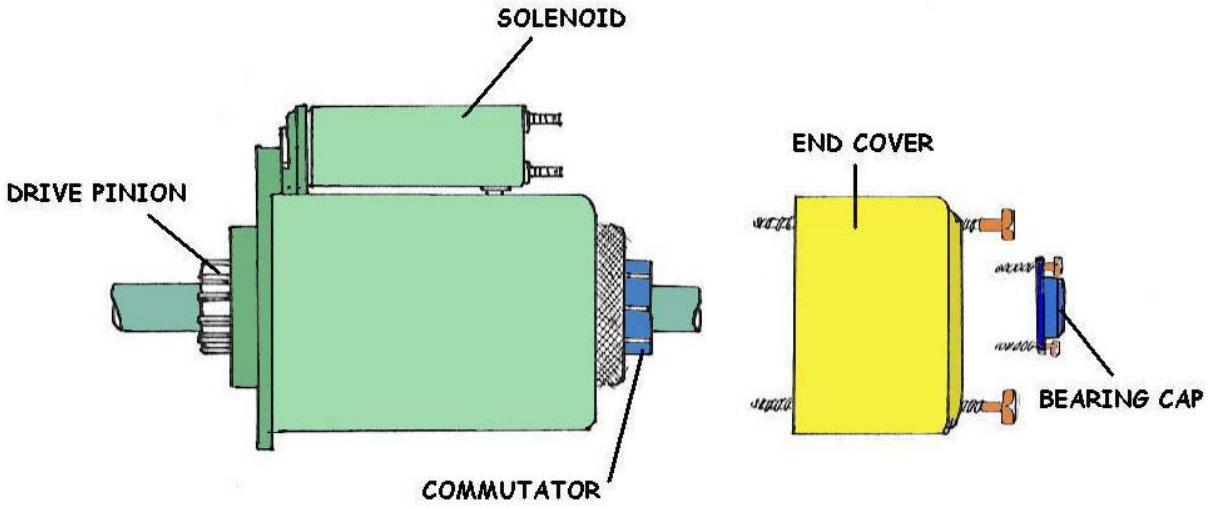
Modern DC brushless motors range in power from a fraction of a watt to many kilowatts. Larger brushless motors up to about 100 kW rating are used in electric vehicles. They also find significant use in high-performance electric model aircraft.

Coreless DC Motors
Nothing in the design of any of the motors described above requires that the iron (steel) portions of the rotor actually rotate; torque is exerted only on the windings of the electromagnets. Taking advantage of this fact is the coreless DC motor, a specialized form of a brush or brushless DC motor. Optimized for rapid acceleration, these motors have a rotor that is constructed without any iron core.

The rotor can take the form of a winding-filled cylinder inside the stator magnets, a basket surrounding the stator magnets, or a flat pancake (possibly formed on a printed wiring board) running between upper and lower stator magnets. The windings are typically stabilized by being impregnated with electrical epoxy potting systems. Filled epoxies that have moderate mixed viscosity and a long gel time. These systems are highlighted by low shrinkage and low exotherm.

Because the rotor is much lighter in weight (mass) than a conventional rotor formed from copper windings on steel laminations, the rotor can accelerate much more rapidly, often achieving a mechanical time constant under 1 ms. This is especially true if the windings use aluminum rather than the heavier copper.

But because there is no metal mass in the rotor to act as a heat sink, even small coreless motors must often be cooled by forced air. These motors were commonly used to drive the capstan(s) of magnetic tape drives and are still widely used in high-performance servo-controlled systems, like radio-controlled vehicles/aircraft, humanoid robotic systems, industrial automation, medical devices, etc.



STARTER MOTOR

Universal Motors

A variant of the wound field DC motor is the universal motor. The name derives from the fact that it may use AC or DC supply current, although in practice they are nearly always used with AC supplies. The principle is that in a wound field DC motor the current in both the field and the armature (and hence the resultant magnetic fields) will alternate (reverse polarity) at the same time, and hence the mechanical force generated is always in the same direction. In practice, the motor must be specially designed to cope with the AC current (impedance must be taken into account, as must the pulsating force), and the resultant motor is generally less efficient than an equivalent pure DC motor. Operating at normal power line frequencies, the maximum output of universal motors is limited and motors exceeding one kilowatt are rare. But universal motors also form the basis of the traditional railway traction motor in electric railways. In this application, to keep their electrical efficiency high, they were operated from very low frequency AC supplies, with 25 Hz and 16 2/3 hertz operation being common. Because they are universal motors, locomotives using this design were also commonly capable of operating from a third rail powered by DC.

The advantage of the universal motor is that AC supplies may be used on motors which have the typical characteristics of DC motors, specifically high starting torque and very compact design if high running speeds are used. The negative aspect is the maintenance and short life problems caused by the commutator. As a result, such motors are usually used in AC devices such as food mixers and power tools, which are used only intermittently. Continuous speed control of a universal motor running on AC is very easily accomplished using a thyristor circuit, while stepped speed control can be accomplished using multiple taps on the field coil.

Household blenders that advertise many speeds frequently combine a field coil with several taps and a diode that can be inserted in series with the motor (causing the motor to run on half-wave rectified AC).

Universal motors can rotate at relatively high revolutions per minute (rpm). This makes them useful for appliances such as blenders, vacuum cleaners, and hair dryers where high-speed operation is desired. Many vacuum cleaner and weed trimmer motors exceed 10,000 rpm; Dremel and other similar miniature grinders will often exceed 30,000 rpm. Motor damage may occur due to overspeed (rpm in excess of design specifications) if the unit is operated with no significant load. On larger motors, sudden loss of load is to be avoided, and the possibility of such an occurrence is incorporated into the motor's protection and control schemes. Often, a small fan blade attached to the armature acts as an artificial load to limit the motor speed to a safe value, as well as provide cooling airflow to the armature and field windings. With the very low cost of semiconductor rectifiers, some applications that would have previously used a universal motor now use a pure DC motor, sometimes with a permanent magnet field.

AC Motors

In 1882, Nicola Tesla identified the rotating magnetic field principle, and pioneered the use of a rotary field of force to operate machines. He exploited the principle to design a unique two-phase induction motor in 1883. In 1885, Galileo Ferraris independently researched the concept. In 1888, Ferraris published his research in a paper to the Royal Academy of Sciences in Turin.

Introduction of Tesla's motor from 1888 onwards initiated what is sometimes referred to as the Second Industrial Revolution, making possible the efficient generation and long distance distribution of electrical energy using the alternating current transmission system, also of Tesla's invention (1888). Before the invention of the rotating magnetic field, motors operated by continually passing a conductor through a stationary magnetic field (as in homopolar motors). Tesla had suggested that the commutators from a machine could be removed and the device could operate on a rotary field of force. Professor Poeschel, his teacher, stated that would be akin to building a perpetual motion machine.

Components

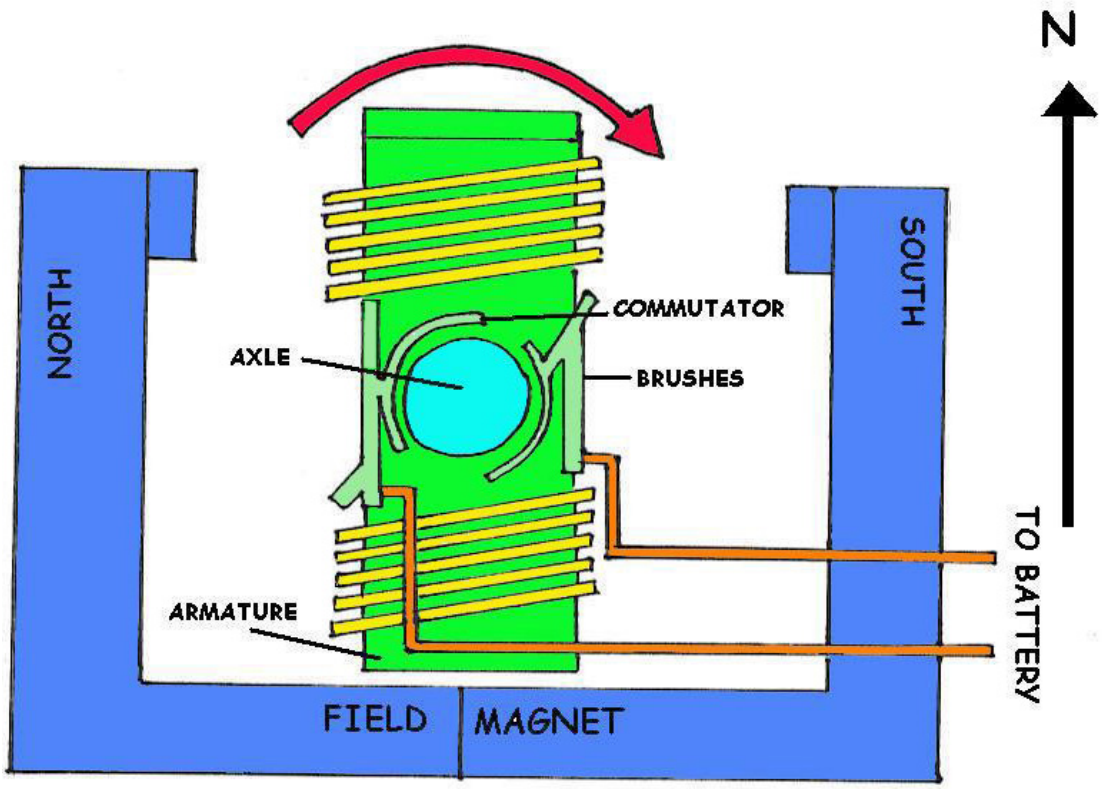
A typical AC motor consists of two parts:

1. An outside stationary stator having coils supplied with AC current to produce a rotating magnetic field, and;
2. An inside rotor attached to the output shaft that is given a torque by the rotating field.

Torque Motors

A torque motor is a specialized form of induction motor which is capable of operating indefinitely at stall (with the rotor blocked from turning) without damage. In this mode, the motor will apply a steady stall torque to the load (hence the name).

A common application of a torque motor would be the supply- and take-up reel motors in a tape drive. In this application, driven from a low voltage, the characteristics of these motors allow a relatively-constant light tension to be applied to the tape whether or not the capstan is feeding tape past the tape heads. Driven from a higher voltage, (and so delivering a higher torque), the torque motors can also achieve fast-forward and rewind operation without requiring any additional mechanics such as gears or clutches. In the computer world, torque motors are used with force feedback steering wheels.



Slip Ring

The slip ring or wound rotor motor is an induction machine where the rotor comprises a set of coils that are terminated in slip rings to which external impedances can be connected. The stator is the same as is used with a standard squirrel cage motor. By changing the impedance connected to the rotor circuit, the speed/current and speed/torque curves can be altered.

The slip ring motor is used primarily to start a high inertia load or a load that requires a very high starting torque across the full speed range. By correctly selecting the resistors used in the secondary resistance or slip ring starter, the motor is able to produce maximum torque at a relatively low current from zero speed to full speed. A secondary use of the slip ring motor is to provide a means of speed control.

Because the torque curve of the motor is effectively modified by the resistance connected to the rotor circuit, the speed of the motor can be altered. Increasing the value of resistance on the rotor circuit will move the speed of maximum torque down. If the resistance connected to the rotor is increased beyond the point where the maximum torque occurs at zero speed, the torque will be further reduced. When used with a load that has a torque curve that increases with speed, the motor will operate at the speed where the torque developed by the motor is equal to the load torque. Reducing the load will cause the motor to speed up, and increasing the load will cause the motor to slow down until the load and motor torque are equal. Operated in this manner, the slip losses are dissipated in the secondary resistors and can be very significant. The speed regulation is also very poor.

Stepper Motors

Closely related in design to three-phase AC synchronous motors are stepper motors, where an internal rotor containing permanent magnets or a large iron core with salient poles is controlled by a set of external magnets that are switched electronically. A stepper motor may also be thought of as a cross between a DC electric motor and a solenoid.

As each coil is energized in turn, the rotor aligns itself with the magnetic field produced by the energized field winding. Unlike a synchronous motor, in its application, the motor may not rotate continuously; instead, it "steps" from one position to the next as field windings are energized and de-energized in sequence. Depending on the sequence, the rotor may turn forwards or backwards.

Simple stepper motor drivers entirely energize or entirely de-energize the field windings, leading the rotor to "cog" to a limited number of positions; more sophisticated drivers can proportionally control the power to the field windings, allowing the rotors to position between the cog points and thereby rotate extremely smoothly. Computer controlled stepper motors are one of the most versatile forms of positioning systems, particularly when part of a digital servo-controlled system.

Stepper motors can be rotated to a specific angle with ease, and hence stepper motors are used in pre-gigabyte era computer disk drives, where the precision they offered was adequate for the correct positioning of the read/write head of a hard disk drive. As drive density increased, the precision limitations of stepper motors made them obsolete for hard drives, thus newer hard disk drives use read/write head control systems based on voice coils. Stepper motors were upscaled to be used in electric vehicles under the term SRM (switched reluctance machine).

Linear Motors
A linear motor is essentially an electric motor that has been "unrolled" so that, instead of producing a torque (rotation), it produces a linear force along its length by setting up a traveling electromagnetic field. Linear motors are most commonly induction motors or stepper motors. You can find a linear motor in a maglev (Transrapid) train, where the train "flies" over the ground and in many roller-coasters where the rapid motion of the motor-less railcar is controlled by the rail.

Doubly-fed Electric Motor
Doubly-fed electric motors have two independent multiphase windings that actively participate in the energy conversion process with at least one of the winding sets electronically controlled for variable speed operation. Two is the most active multiphase winding sets possible without duplicating singly-fed or doubly-fed categories in the same package. As a result, doubly-fed electric motors are machines with an effective constant torque speed range that is twice synchronous speed for a given frequency of excitation. This is twice the constant torque speed range as singly-fed electric machines, which have only one active winding set.


WHERE'S THE MOTOR?

Coupling Section

The pump coupling serves two main purposes:

- It couples or joins the two shafts together to transfer the rotation from motor to impeller.
- It compensates for small amounts of misalignment between the pump and the motor.

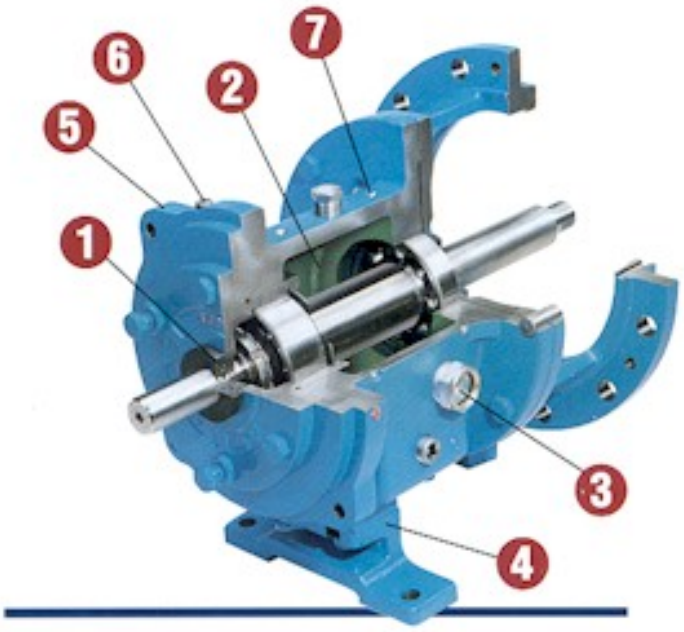
Remember that any coupling is a device in motion. If you have a 4-inch diameter coupling rotating at 1800 rpm, its outer surface is traveling about 20 mph. With that in mind, can you think of safety considerations?

There are three commonly used types of couplings: ***Rigid, Flexible and V-belts.***

Rigid Coupling
Rigid couplings are most commonly used on vertically mounted pumps. The rigid coupling is usually specially keyed or constructed for joining the coupling to the motor shaft and the pump shaft. There are two types of rigid couplings: the flanged coupling, and the split coupling.

Flexible Coupling. The flexible coupling provides the ability to compensate for small shaft misalignments. Shafts should be aligned as close as possible, regardless. The greater the misalignment, the shorter the life of the coupling. Bearing wear and life are also affected by misalignment.

1. Oil Seals
2. Large Oil Sump
3. Bulls Eye Sight Glass
4. Rigid Frame Foot
5. C-Face Mounting Flange
6. Lubrication Flexibility
7. Condition Monitoring Sites



Alignment of Flexible and Rigid Couplings

Both flexible and rigid couplings must be carefully aligned before they are connected. Misalignment will cause excessive heat and vibration, as well as bearing wear. Usually, the noise from the coupling will warn you of shaft misalignment problems.

Three types of shaft alignment problems are shown in the pictures below:

ANGULAR MISALIGNMENT

ANGULAR AND PARALLEL

PARALLEL MISALIGNMENT

Different couplings will require different alignment procedures. We will look at the general procedures for aligning shafts.

1. Place the coupling on each shaft.
2. Arrange the units so they appear to be aligned. (Place shims under the legs of one of the units to raise it.)
3. Check the run-out, or difference between the driver and driven unit, by rotating the shafts by hand.
4. Turn both units so that the maximum run-out is on top.

Now you can check the units for both parallel and angular alignment. Many techniques are used, such as: straight edge, needle deflection (dial indicators), calipers, tapered wedges, and Laser alignment.

V-Belt Drive Couplings
V-belt drives connect the pump to the motor. A pulley is mounted on the pump and motor shaft. One or more belts are used to connect the two pulleys. Sometimes a separately mounted third pulley is used. This idler pulley is located off centerline between the two pulleys, just enough to allow tensioning of the belts by moving the idler pulley. An advantage of driving a pump with belts is that various speed ratios can be achieved between the motor and the pump.

Shaft Bearings
There are three types of bearings commonly used: ball bearings, roller bearings, and sleeve bearings. Regardless of the particular type of bearings used within a system--whether it is ball bearings, a sleeve bearing, or a roller bearing--the bearings are designed to carry the loads imposed on the shaft.

Bearings must be lubricated. Without proper lubrication, bearings will overheat and seize. Proper lubrication means using the correct type and the correct amount of lubrication. Similar to motor bearings, shaft bearings can be lubricated either by oil or by grease.

How can we prevent the water from leaking along the shaft?
A special seal is used to prevent liquid leaking out along the shaft. There are two types of seals commonly used:

- **Packing seal**
- **Mechanical seal**

Packing Seals

Should packing have leakage?

Leakage
During pump operation, a certain amount of leakage around the shafts and casings normally takes place.

This leakage must be controlled for two reasons: (1) to prevent excessive fluid loss from the pump, and (2) to prevent air from entering the area where the pump suction pressure is below atmospheric pressure.

The amount of leakage that can occur without limiting pump efficiency determines the type of shaft sealing selected. Shaft sealing systems are found in every pump. They can vary from simple packing to complicated sealing systems.

Packing is the most common and oldest method of sealing. Leakage is checked by the compression of packing rings that causes the rings to deform and seal around the pump shaft and casing. The packing is lubricated by liquid moving through a lantern ring in the center of the packing. The sealing slows down the rate of leakage. It does not stop it completely, since a certain amount of leakage is necessary during operation. Mechanical seals are rapidly replacing conventional packing on centrifugal pumps.

Some of the reasons for the use of mechanical seals are as follows:
1. Leaking causes bearing failure by contaminating the oil with water. This is a major problem in engine-mounted water pumps.

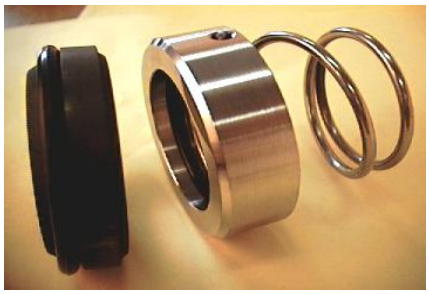
2. Properly installed mechanical seals eliminate leakoff on idle (vertical) pumps. This design prevents the leak (water) from bypassing the water flinger and entering the lower bearings.

Leakoff causes two types of seal leakage:
a. Water contamination of the engine lubrication oil.
b. Loss of treated fresh water that causes scale buildup in the cooling system.

Centrifugal pumps are versatile and have many uses. This type of pump is commonly used to pump all types of water and wastewater flows, including thin sludge.

Lantern Rings
Lantern rings are used to supply clean water along the shaft. This helps to prevent grit and air from reaching the area. Another component is the slinger ring. The slinger ring is an important part of the pump because it is used to protect the bearings. Other materials can be used to prevent this burier.

Mechanical Seals
Mechanical seals are commonly used to reduce leakage around the pump shaft. There are many types of mechanical seals. The photograph below illustrates the basic components of a mechanical seal. Similar to the packing seal, clean water is fed at a pressure greater than that of the liquid being pumped.



There is little or no leakage through the mechanical seal. The wearing surface must be kept extremely clean. Even fingerprints on the wearing surface can introduce enough dirt to cause problems.

What care should be taken when storing mechanical seals?

Mechanical Seals

Wear Rings
Not all pumps have wear rings. However, when they are included, they are usually replaceable. Wear rings can be located on the suctions side and head side of the volute. Wear rings could be made of the same metal but of different alloys. The wear ring on the head side is usually a harder alloy.

It's called a ***"WEAR RING"*** and what would be the purpose?

Mechanical Seals

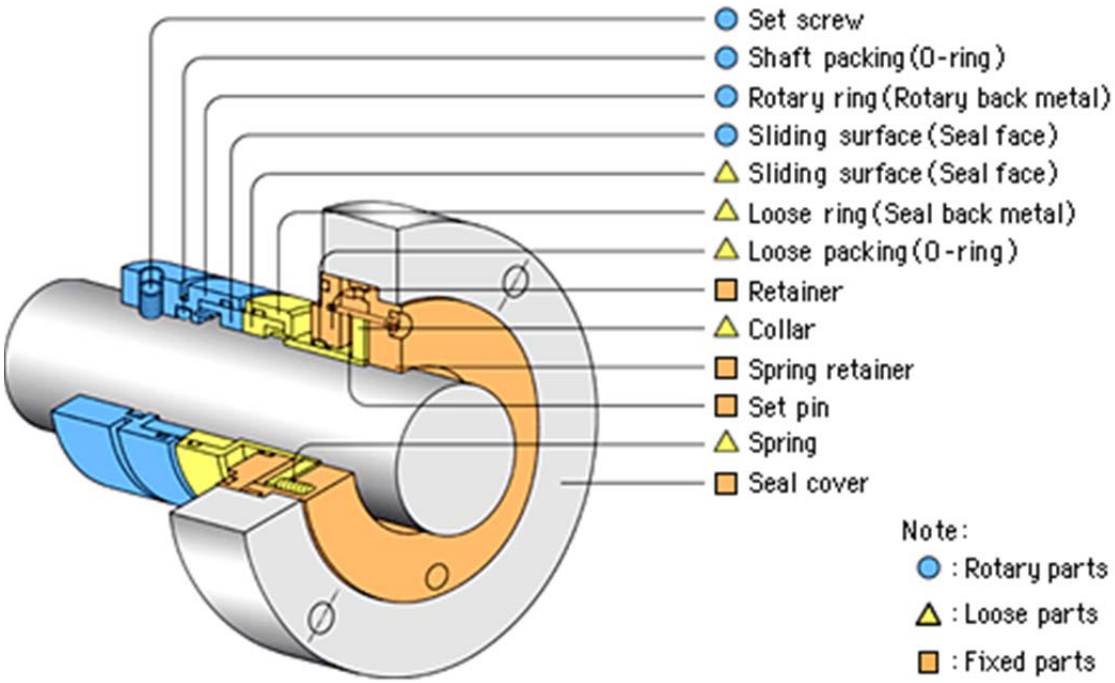
Mechanical seals are rapidly replacing conventional packing as the means of controlling leakage on rotary and positive-displacement pumps. Mechanical seals eliminate the problem of excessive stuffing box leakage, which causes failure of pump and motor bearings and motor windings.

Mechanical seals are ideal for pumps that operate in closed systems (such as fuel service and air-conditioning, chilled-water, and various cooling systems). They not only conserve the fluid being pumped, but also improve system operation.

The type of material used for the seal faces will depend upon the service of the pump. Most water service pumps use a carbon material for one of the seal faces and ceramic (tungsten carbide) for the other. When the seals wear out, they are simply replaced.

You should replace a mechanical seal whenever the seal is removed from the shaft for any reason, or whenever leakage causes undesirable effects on equipment or surrounding spaces. Do not touch a new seal on the sealing face because body acid and grease or dirt will cause the seal to pit prematurely and leak.

Mechanical shaft seals are positioned on the shaft by stub or step sleeves. Mechanical shaft seals must not be positioned by setscrews. Shaft sleeves are chamfered (beveled) on the outboard ends for easy mechanical seal mounting. Mechanical shaft seals serve to ensure that position liquid pressure is supplied to the seal faces under all conditions of operation. They also ensure adequate circulation of the liquid at the seal faces to minimize the deposit of foreign matter on the seal parts.



Maintenance of Centrifugal Pumps

When properly installed, maintained and operated, centrifugal pumps are usually trouble-free. Some of the most common corrective maintenance actions that you may be required to perform are discussed in the following sections.

Repacking - Lubrication of the pump packing is extremely important. The quickest way to wear out the packing is to forget to open the water piping to the seals or stuffing boxes. If the packing is allowed to dry out, it will score the shaft. When operating a centrifugal pump, be sure there is always a slight trickle of water coming out of the stuffing box or seal. How often the packing in a centrifugal pump should be renewed depends on several factors, such as the type of pump, condition of the shaft sleeve, and hours in use.

To ensure the longest possible service from pump packing, make certain the shaft or sleeve is smooth when the packing is removed from a gland. Rapid wear of the packing will be caused by roughness of the shaft sleeve (or shaft where no sleeve is installed). If the shaft is rough, it should be sent to the machine shop for a finishing cut to smooth the surface. If it is very rough, or has deep ridges in it, it will have to be renewed. It is absolutely necessary to use the correct packing. When replacing packing, be sure the packing fits uniformly around the stuffing box. If you have to flatten the packing with a hammer to make it fit, **YOU ARE NOT USING THE RIGHT SIZE.** Pack the box loosely, and set up the packing gland lightly. Allow a liberal leak-off for stuffing boxes that operate above atmospheric pressure.

Next, start the pump. Let it operate for about 30 minutes before you adjust the packing gland for the desired amount of leak-off. This gives the packing time to run-in and swell. You may then begin to adjust the packing gland. Tighten the adjusting nuts one flat at a time. Wait about 30 minutes between adjustments. Be sure to tighten the same amount on both adjusting nuts. If you pull up the packing gland unevenly (or cocked), it will cause the packing to overheat and score the shaft sleeves. Once you have the desired leak-off, check it regularly to make certain that sufficient flow is maintained.

Mechanical Seals

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The type of material used for the seal faces will depend upon the service of the pump. Most water service pumps use a carbon material for one of the seal faces and ceramic (tungsten carbide) for the other. When the seals wear out, they are simply replaced. You should replace a mechanical seal whenever the seal is removed from the shaft for any reason, or whenever leakage causes undesirable effects on equipment or surrounding spaces. Do not touch a new seal on the sealing face because body acid and grease or dirt will cause the seal to pit prematurely and leak.

Pumping and Lift Station Chapter Highlights

Pump Stations
Proper operation, maintenance, and repair of pump stations typically requires special electrical, hydraulic, and mechanical knowledge. Pump station failure may damage equipment, the environment, or endanger public health. Variation in equipment types, pump station configuration, and geographical factors determine pump station design and O&M requirements.

The reviewer should verify that the O&M manual contains procedures in writing for the following:

• Are pumps rotated manually or automatically? If manually, how frequently?

• Are wet well operating levels set to limit pump starts and stops?

• Is there a procedure for manipulating pump operations (manually or automatically) during wet weather to increase in-line storage of wet weather flows?

• Is flow monitoring provided? How is the collected data used?

• Does the pump station have capacity-related overflows? Maintenance related overflows? Is overflow monitoring provided?

• Is there a history of power outages? Is there a source of emergency power? If the emergency power source is a generator, is it regularly exercised under load?

Operation and Maintenance (O&M) Activities
Proactive O&M initiatives are critical to effective prevention of SSOs. Nationwide, improved O&M activities such as implementation of hot spot cleaning programs, routine pipeline cleaning, and video inspections to find structural deficiencies have dramatically reduced the frequency and severity of SSOs in many cities. Your system should conduct various types of proactive O&M activities throughout their service area.

Suggested goals of your system's wastewater collection system maintenance programs should be as follows:

- Maintain wastewater collection system flow capacity.
- Reduce the frequency and duration of overflow events.
- Optimize the use of resources.
- Optimize the life cycle of system components.
- Maintain accurate maintenance records.

Your section of the CMOM Plan shall include descriptions of maintenance facilities, mapping and data management, routine O&M activities, system repairs, and training.

Maintenance Program
Every collection system owner or operator should have a well-planned, systematic, and comprehensive maintenance program. The goals of a maintenance program should include:
• Prevention of overflows.
• Maximization of service and system reliability at minimum cost.
• Assurance of infrastructure sustainability (i.e., ensure all components reach their service life).

There should then be procedures which describe the maintenance approach for various systems. In addition, there should be detailed instructions for the maintenance and repair of individual facilities. These instructions should provide a level of detail such that any qualified collection system personnel or repair technician could perform the repair or maintenance activity.

Maintenance may be planned or unplanned. There are essentially two types of planned maintenance; predictive and preventive. Predictive maintenance is a method that tries to look for early warning signs of equipment failure such that emergency maintenance is avoided.

Preventive maintenance consists of scheduled maintenance activities performed on a regular basis. There are two types of unplanned maintenance, corrective and emergency. Corrective maintenance consists of scheduled repairs to problems identified under planned or predictive maintenance.

Emergency maintenance is activities (typically repairs) performed in response to a serious equipment or line failure where action must be taken immediately. The goal of every owner or operator should be to reduce corrective and emergency maintenance through the use of planned and predictive maintenance. The reviewer should evaluate the progress of the owner or operator in achieving that goal.

The goals of the reviewer in assessment of the maintenance program are:
• Identify SSOs caused by inadequate maintenance.
• Determine maintenance trends (i.e., frequent emergency maintenance performed as opposed to predictive maintenance.)
• Identify sustainability issues (i.e., inadequate maintenance to allow system components to reach service life and/or many components nearing or at service life.)

Pump Station Inspection
Pump stations should be subject to inspection and preventive maintenance on a regular schedule. The frequency of inspection may vary from once a week, for a reliable pump station equipped with a telemetry system, to continuous staffing at a large pump station.

The basic inspection should include verification that alarm systems are operating properly, wet well levels are properly set, all indicator lights and voltage readings are within acceptable limits, suction and discharge pressures are within normal limits, that the pumps are running without excessive heat or vibration and have the required amount of lubrication, and that the emergency generator is ready if needed. Less frequent inspections may include such items as vibration analysis and internal inspection of pump components.

Pump Station Checklist
Observations and tasks performed should be recorded in a log book or on a checklist at the pump station. It is important to note how this data returns to the central maintenance data management system. At the time of the inspection, collection system personnel may perform minor repairs if necessary. If non-emergency repairs are required that are beyond the staff's training, it will probably be necessary to prepare a work order which routes a request though the proper channels to initiate the repair action. During the review the reviewer should check a random number of work orders to see how they move through the system. The reviewer should note whether repairs are being carried out promptly. In pump stations, for critical equipment (pumps, drives, power equipment, and control equipment), there should not be much backlog, unless the staff is waiting for parts.

During the review, the reviewer should also make on-site observations of a representative pump stations. The reviewer should plan at least half an hour to look at the simplest two-pump prefabricated station, and one to two hours to look at a larger station. In large systems, drive time between stations may be significant.

The reviewer should strive to see a range of pump station sizes and types (i.e., the largest, smallest, most remote, and any that review of work orders has indicated might be problematic).

Overall, the pump station should be clean, in good structural condition, and exhibit minimal odor. The reviewer should note the settings of the pumps (i.e., which are operating, which are on stand-by, and which are not operating and why). The operating pumps should be observed for noise, heat, and excessive vibration. The settings in the wet well should be noted (as indicated on the controls, as direct observation of the reviewer in the wet well is not recommended) and the presence of any flashing alarm lights.

Atmospheric Hazards
The reviewer is reminded of the atmospheric hazards in a pump station (make sure ventilation has been running prior to arrival) and to avoid confined space entry. If the pump station has an overflow its outlet should be observed, if possible, for signs of any recent overflows such as floatable materials or toilet paper. The reviewer should check the log book and/or checklist kept at the pump station to ensure that records are current and all maintenance activities have been performed. Below is a listing of items that indicate inadequate maintenance:

- Overall poor housekeeping and cleanliness.
- Excessive grease accumulation in wet well.
- Excessive corrosion on railings, ladders, and other metal components.
- Sagging, worn, improperly sized, or inadequate belts.
- Excessive equipment out of service for repair or any equipment for which repair has not been ordered (i.e., a work order issued.)
- Pumps running with excessive heat, vibration, or noise.
- Peeling paint and/or dirty equipment (the care given to equipment's outer surfaces often, but not always, mirrors internal condition.)
- Check valves not closing when pumps shut off.
- Inoperative instrumentation, alarms, and recording equipment.
- "Jury-rigged" repairs (i.e., "temporary" repairs using inappropriate materials.)
- Leakage from pumps, piping, or valves (some types of pump seals are designed to "leak" seal water.)
- Inadequate lighting or ineffective/inoperative ventilation equipment.

Routine Preventative O&M Activities – Wastewater Lift Stations and Force Mains
Perform Regular Preventative Maintenance
The wastewater collections service technicians should perform regular preventative maintenance on the various components at the lift stations. An outside contractor may also be used to clean each lift station twice a year.

Most wastewater lift station and force main operations are typically remotely monitored and controlled through a dial-up telemetry system that sends signals to the system's operation center. In the event of a malfunction, all of the lift stations have redundant pump and pump monitoring systems, and all but six have emergency backup power generation.

System Repairs
Deficiencies in the sewer system requiring repair are noted during cleaning and video inspections or are discovered through investigation of customer complaints. A Supervisor should make arrangements for all repairs; small repairs are often completed by the system's crews and larger repairs are completed by a qualified outside contractor.

Deficiencies in lift stations and force mains requiring repair should be noted by the wastewater collections technicians during their routine visits, by alarms or through customer complaints. The Supervisor should make arrangements for all lift station and force main repairs.

Maintenance Budgeting

The cost of a maintenance program is a significant part of the annual operating budget. The collection system owner or operator should track all maintenance costs incurred throughout the year, both by internal staff and contractors, to ensure that the budget is based on representative costs from past years. Budgets should be developed from past cost records which usually are categorized according to preventive maintenance, corrective maintenance, and projected and actual major repair requirements. Annual costs should be compared to the budget periodically to control maintenance expenditures.

The reviewer should evaluate the maintenance budget, keeping in mind the system's characteristics, such as age. Costs for emergency repairs should be a relatively small percentage of the budget--five to ten percent would not be considered excessive.

The establishment of an "emergency reserve" may also be included as part of the maintenance budget. This is especially useful where full replacement is not funded.

The budget should also be considered in light of maintenance work order backlog.

Planned and Unplanned Maintenance

A planned maintenance program is a systematic approach to performing maintenance activities so that equipment failure is avoided. Planned maintenance is composed of predictive and preventive maintenance. In the end, a good planned maintenance program should reduce material, capital repair, and replacement costs, improve personnel utilization and morale, reduce SSOs, and sustain public confidence.

Examples of predictive maintenance includes monitoring equipment for early warning signs of impending failure, such as excess vibration, heat, dirty oil, and leakage. Assessment and inspection activities can be classified as predictive maintenance.

Vibration and lubrication analyses, thermography, and ultrasonics are among the more common predictive maintenance tools.

Predictive maintenance also takes into account historical information about the system as all systems will deteriorate over time. A predictive maintenance program strives to identify potential problem areas and uncover trends that could affect equipment performance.

Predictive maintenance offers an early warning. It allows collection system personnel to detect early signs of increasing rates of wear and therefore failure, and thus shift a "corrective" task into a "planned" task. To be truly effectively predictive, however, maintenance should not spur personnel into doing the work too soon and wasting useful life and value of the equipment in question.

The reviewer should inquire as to whether tools such as vibration and lubrication analysis, thermography, or ultrasonics are used, and obtain information on the extent of the programs.

The basis of a good predictive maintenance program is recordkeeping. Only with accurate recordkeeping can baseline conditions be established, problem areas identified, and a proactive approach taken to repairs and replacement.

Effective preventive maintenance minimizes system costs and environmental impacts by reducing breakdowns and thus the need for corrective or emergency maintenance; improves reliability by minimizing the time equipment is out of service; increases the useful life of equipment, thus avoiding costly premature replacement; and avoids potential noncompliance situations.

An Effective Preventive Maintenance Program Includes:

- Trained personnel.
- Scheduling based on system specific knowledge.
- Detailed instructions related to the maintenance of various pieces of equipment.
- A system for recordkeeping.
- System knowledge in the form of maps, historical knowledge and records. An effective preventive maintenance program builds on the inspection activities and predictive maintenance described above, and includes a well thought-out schedule for these activities.

The basis of the schedule for mechanical equipment maintenance (i.e., pump station components) should be the manufacturers' recommended activities and frequencies. This schedule may then be augmented by the knowledge and experience of collection system personnel to reflect the site-specific requirements. The schedule for sewer line cleaning, inspection, root removal, and repair activities should be based on periodic inspection data. In most systems, uniform frequencies for sewer line cleaning, inspection, and root removal are not necessary and inefficient. In many systems, a relatively small percentage of the pipe generates most of the problems.

Efficient use of inspection data allows the owner or operator to implement a schedule in the most constructive manner. In rare cases it may be appropriate to reduce maintenance frequency for a particular piece of equipment.

Lubrication
Lubrication is probably one of the most important maintenance activities for mechanical systems, such as pumps and motors. Frequencies of lubrication, choice of lubricant and lubrication procedure are all important factors in this activity. These items should closely follow manufacturer instructions, but may be modified to fit site-specific conditions and particular equipment applications. An example of a scheduling code and maintenance schedule for a pump is shown below:

Guide for Evaluating CMOM Programs at Sanitary Sewer Collection Systems

Rotary Pump Maintenance Schedule

Frequency	Maintenance Required
D	Check packing gland assembly
D	Check discharge pressure
S	Inspect and lubricate bearings
A	Flush bearings and replace lubricant

D = Daily A = Annually S = Semiannually

Typically, there is a maintenance card or record for each piece of equipment within the collection system. These records should contain maintenance recommendations, schedule, and instructions on conducting the specific maintenance activity. The records should include documentation regarding any maintenance activities conducted to date and other observations related to that piece of equipment or system. Maintenance records are generally kept where maintenance personnel have easy access to them. The reviewer should examine the full series of periodic work orders (i.e. weekly, monthly, semiannually, and annually) for a selection of system components (e.g., a few pump stations, several line segments).

The reviewer should then compare the recommended maintenance frequency to that which is actually performed. He or she should also look at the backlog of work; not focusing solely on the number of backlogged work orders, but on what that number represents in time.

A very large system can have a hundred orders backlogged and only be one week behind. In a computerized system, a listing of all open work orders is usually very simple for collection system personnel to generate. The owner or operator should be able to explain their system for prioritizing work orders.

The reviewer needs to clearly understand the following:
• How the maintenance data management system works
• How work orders are generated and distributed
• How field crews use the work orders
• How data from the field is collected and returned
• How and on whose authority work orders are closed out

The reviewer should check to see if data entry is timely and up to date.

Unplanned Maintenance
Unplanned maintenance is that which takes place in response to equipment breakdowns or emergencies. Unplanned maintenance may be corrective or emergency maintenance. Corrective maintenance could occur as a result of preventive or predictive maintenance activities which identified a problem situation.

A work order should be issued so that the request for corrective maintenance is directed to the proper personnel. An example of non-emergency corrective maintenance could be a broken belt on a belt driven pump. The worn belt was not detected and replaced through preventive maintenance and therefore the pump is out of service until corrective maintenance can be performed. Although the pump station may function with one pump out of service, should another pump fail, the situation may become critical during peak flow periods. If the information can be easily generated the reviewer should select a sampling of work orders and compare them to the corrective maintenance database to determine if repairs are being made in a timely manner. Reviewers should note the current backlog of corrective maintenance work orders. A corrective maintenance backlog of two weeks or less would indicate an owner or operator in control of corrective maintenance. The owner or operator should be able to explain corrective maintenance work orders that have not been completed within six months.

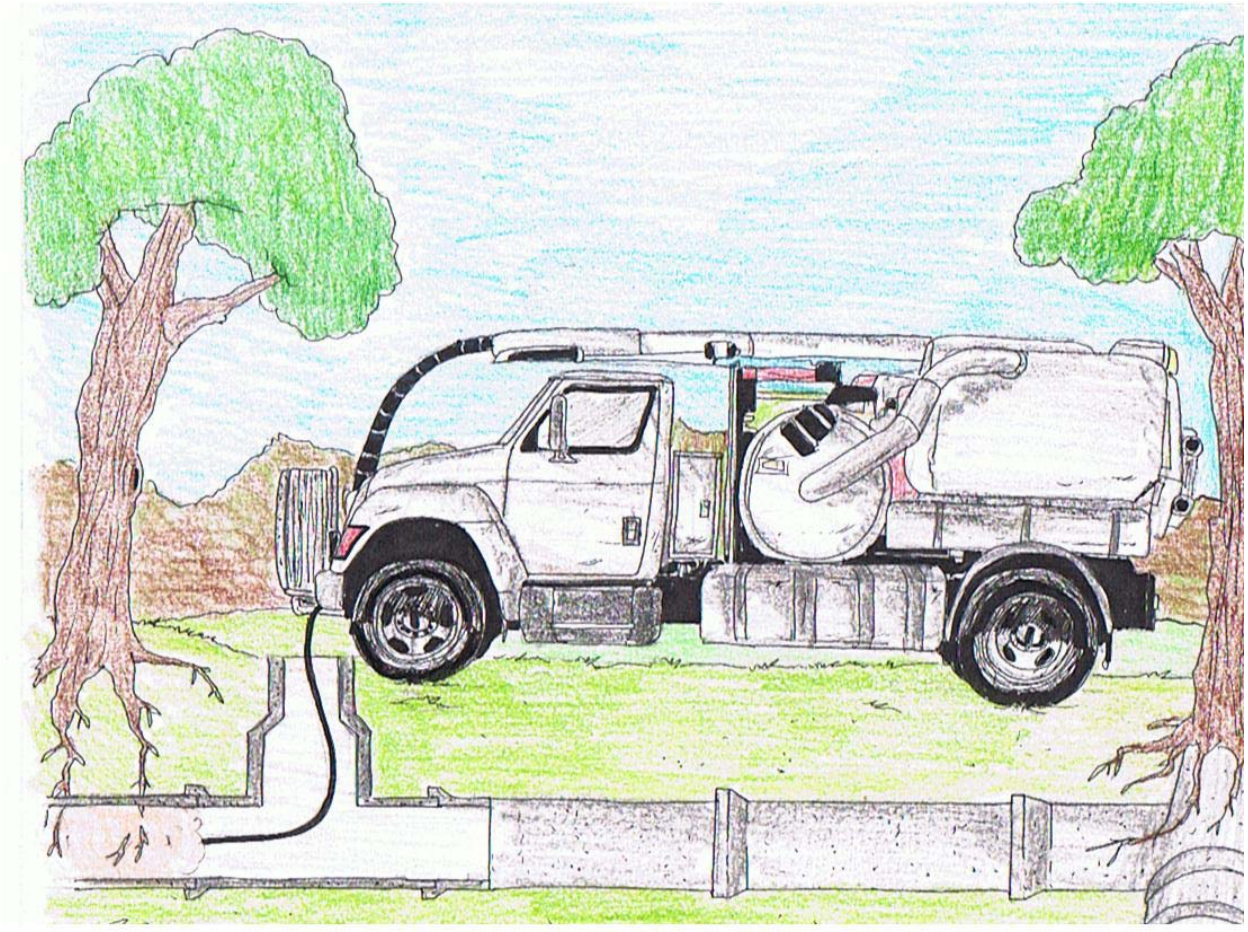
Corrective Maintenance
Corrective maintenance takes resources away from predictive and preventive maintenance. When corrective maintenance becomes a predominant activity, personnel may not be able to perform planned maintenance, thus leading to more corrective maintenance and emergency situations. Emergency maintenance occurs when a piece of equipment or system fails, creating a threat to public health, the environment, or associated equipment. This type of maintenance involves repairs on short notice, of malfunctioning equipment or sewers. A broken force main, totally non-functional pump station and street cave-ins are all examples of emergency situations.

Types of Portable Emergency Equipment
- Bypass pumps
- Portable generator
- Air compressor, trailer-mounted
- Manhole lifters and gas testing equipment
- Sewer rodder and/or flushing machine
- Portable lights and hand tools
- Chemical spray units (for insects and rodent control)
- Truck (1-ton) and trailers
- Vacuum truck
- Repair equipment for excavation (backhoe, shoring equipment, concrete mixers, gasoline operated saws, traffic control equipment, etc.)
- Confined space entry gear

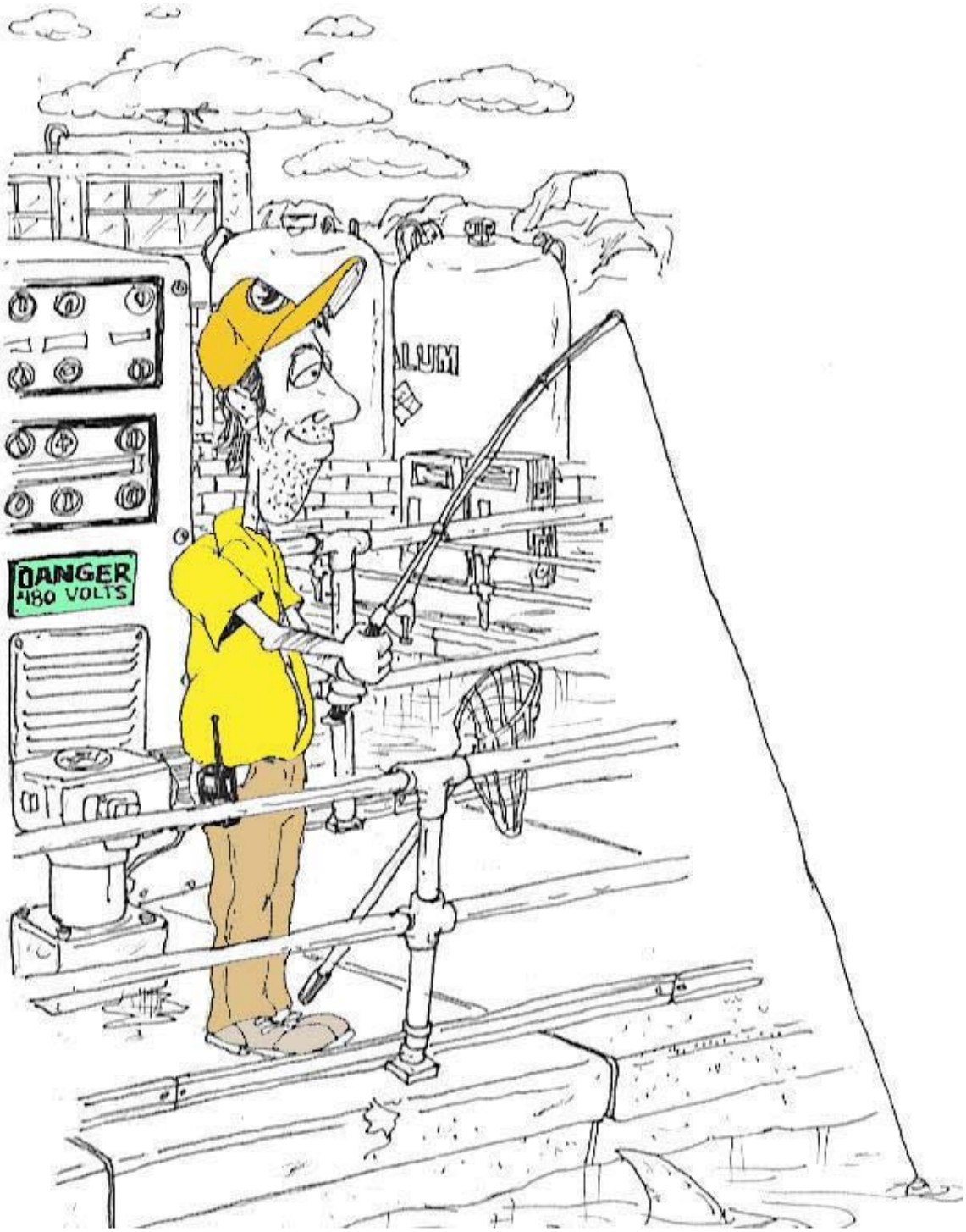
Emergency Crews

Emergency crews should be geared to a 24-hour-a-day, year-round operation. Most large systems have staffed 24-hour crews; many small systems have an “on-call” system. The owner or operator should be able to produce written procedures which spell out the type of action to take in a particular type of emergency and the equipment and personnel requirements necessary to carry out the action.

The crews should have copies of these procedures and be familiar with them. Equipment must be located in an easily accessible area and be ready to move in a short period of time. Vehicles and equipment must be ready to perform, under extreme climatic conditions if necessary. The emergency crew may need materials such as piping, pipe fittings, bedding materials, and concrete. The owner or operator should have supplies on hand to allow for two point (i.e. segment, fitting, or appurtenance) repairs of any part of its system. The reviewer should note the presence of supplies during the review of the yard where equipment and spare parts are maintained and personnel are dispatched.



The best method of controlling hydrogen sulfide is to eliminate its habitat or growth area by keeping sewers cleaner, this will harbor fewer slime bacteria.


DANGER
480 VOLTS

Confined Space Entry Program

Purpose
The Confined Space Entry Program is provided to protect authorized employees that will enter confined spaces and may be exposed to hazardous atmospheres, engulfment in materials, conditions which may trap or asphyxiate due to converging or sloping walls, or contains any other safety or health hazards.

Reference: OSHA-Permit-Required Confined Spaces (**29 CFR 1910.146**).

Scope
You are required to recognize the dangers and hazards associated with confined spaces, and this program is designed to assist you in the safety of and compliance with the OSHA standards associated with such.

Most WWTP's will utilize the Fire Department for all rescues and additional assistance dealing with confined spaces, understanding that most Fire Department operations utilize additional in house SOG's/SOP's pertaining to such operations.

Definitions
Confined space:

- Is large enough or so configured that an employee can bodily enter and perform work.
- Has limited or restricted means for entry or exit (i.e. tanks, vessels, silos, storage bins, hoppers, vaults, and pits are spaces that may have limited means of entry).
- Is not designed for continuous employee occupancy.

Permit required confined space (permit space), is a confined space that has one or more of the following characteristics:

1. Contains or has a potential to contain a hazardous atmosphere.

2. Contains a material that has the potential for engulfing an entrant.

3. Has an internal configuration such that an entrant could be trapped or asphyxiated by inwardly covering walls or by a floor which slopes downward and tapers to a smaller cross-section.

4. Contains any other recognized serious safety or health hazard.

Each Permit-Required Confined Space will be marked "*Confined Space - Entry Permit Required*".

Examples of "***Permit Required Confined Spaces.***" Make sure you comply with these Confined Space rules or face civil and/or criminal charges. Several states have criminally charged Supervisors and Attendants for the actions of the employees in a Confined Space/Permit Required Confined Space. ***Don't risk death or the chance of going to jail in order to speed up your job!***

Confined Space Hazards

Fatalities and injuries constantly occur among construction workers who, during the course of their jobs, are required to enter confined spaces. In some circumstances, these workers are exposed to multiple hazards, any of which may cause bodily injury, illness, or death.

Newspaper and magazine articles abound with stories of workers injured and killed from a variety of atmospheric factors and physical agents. Throughout the construction jobsite, contractors and workers encounter both inherent and induced hazards within confined workspaces.

Inherent Hazards
Inherent hazards, such as electrical, thermal, chemical, mechanical, etc., are associated with specific types of equipment and the interactions among them.

Examples include high voltage (shock or corona discharge and the resulting burns), radiation generated by equipment, defective design, omission of protective features (no provision for grounding non-current-carrying conductive parts), high or low temperatures, high noise levels, and high-pressure vessels and lines (rupturing with resultant release of fragments, fluids, gases, etc.).

Inherent hazards usually cannot be eliminated without degrading the system or equipment, or without making them inoperative. Therefore, emphasis must be placed on hazard control methods.

Induced Hazards
Induced hazards arise, and are induced from, a multitude of incorrect decisions and actions that occur during the actual construction process. Some examples are: omission of protective features, physical arrangements that may cause unintentional worker contact with electrical energy sources, oxygen-deficient atmospheres created at the bottom of pits or shafts, lack of safety factors in structural strength, and flammable atmospheres.

Typical Examples of Confined Workspaces
Following are typical examples of confined workspaces in construction which contain both inherent and induced hazards.

Vaults
A variety of vaults are found on the construction jobsite. On various occasions, workers must enter these vaults to perform a number of functions.

The restricted nature of vaults and their frequently below-grade location can create an assortment of safety and health problems.

Oxygen-Deficient Atmosphere
One of the major problems confronting construction workers while working in vaults is the ever-present possibility of an oxygen-deficient atmosphere.

Explosive or Toxic Gases, Vapors, or Fumes
While working in an electrical vault, workers may be exposed to the build-up of explosive gases such as those used for heating (propane). Welding and soldering produce toxic fumes which are confined in the limited atmosphere.

Electrical Shock
Electrical shock is often encountered from power tools, line cords, etc. In many instances, such electrical shock results from the fact that the contractor has not provided an approved grounding system or the protection afforded by ground-fault circuit interrupters or low-voltage systems.

Purging
In some instances, purging agents such as nitrogen and argon may enter the vault from areas adjacent to it. These agents may displace the oxygen in the vault to the extent that it will asphyxiate workers almost immediately.

Materials Falling In and On
A hazard normally considered a problem associated with confined spaces is material or equipment which may fall into the vault or onto workers as they enter and leave the vault.

Vibration could cause the materials on top of the vault to roll off and strike workers. If the manhole covers were removed, or if they were not installed in the first place, materials could fall into the vault, causing injury to the workers inside.

Condenser Pits
A common confined space found in the construction of nuclear power plants is the condenser pit. Because of their large size, they are often overlooked as potentially hazardous confined spaces. These below-grade areas create large containment areas for the accumulation of toxic fumes, gases, and so forth, or for the creation of oxygen-deficient atmospheres when purging with argon, Freon, and other inert gases. Other hazards will be created by workers above dropping equipment, tools, and materials into the pit.

Manholes
Throughout the construction site, manholes are commonplace. As means of entry into and exit from vaults, tanks, pits, and so forth, manholes perform a necessary function. However, these confined spaces may present serious hazards which could cause injuries and fatalities. A variety of hazards are associated with manholes. To begin with, the manhole could be a dangerous trap into which the worker could fall. Often covers are removed and not replaced, or else they are not provided in the first place.

Pipe Assemblies
One of the most frequently unrecognized types of confined spaces encountered throughout the construction site is the pipe assembly. Piping of sixteen to thirty-six inches in diameter is commonly used for a variety of purposes.

For any number of reasons, workers will enter the pipe. Once inside, they are faced with potential oxygen-deficient atmospheres, often caused by purging with argon or another inert gas. Welding fumes generated by the worker in the pipe, or by other workers operating outside the pipe at either end, subject the worker to toxic atmospheres.

The generally restricted dimensions of the pipe provide little room for the workers to move about and gain any degree of comfort while performing their tasks. Once inside the pipe, communication is extremely difficult. In situations where the pipe bends, communication and extrication become even more difficult. Electrical shock is another problem to which the worker is exposed.

Ungrounded tools and equipment or inadequate line cords are some of the causes. As well, heat within the pipe run may cause the worker to suffer heat prostration.

Ventilation Ducts
Ventilation ducts, like pipe runs, are very common at the construction site. These sheet metal enclosures create a complex network which moves heated and cooled air and exhaust fumes to desired locations in the plant.

Ventilation ducts may require that workers enter them to cut out access holes, install essential parts of the duct, etc. Depending on where these ducts are located, oxygen deficiency could exist. They usually possess many bends, which create difficult entry and exit and which also make it difficult for workers inside the duct to communicate with those outside it. Electrical shock hazards and heat stress are other problems associated with work inside ventilation ducts.

Tanks
Tanks are another type of confined workspace commonly found in construction. They are used for a variety of purposes, including the storage of water, chemicals, etc.

Tanks require entry for cleaning and repairs. Ventilation is always a problem. Oxygen-deficient atmospheres, along with toxic and explosive atmospheres created by the substances stored in the tanks, present hazards to workers. Heat, another problem in tanks, may cause heat prostration, particularly on a hot day.

Since electrical line cords are often taken into the tank, the hazard of electrical shock is always present. The nature of the tank's structure often dictates that workers must climb ladders to reach high places on the walls of the tank.

Sumps
Sumps are commonplace. They are used as collection places for water and other liquids. Workers entering sumps may encounter an oxygen-deficient atmosphere.
Also, because of the wet nature of the sump, electrical shock hazards are present when power tools are used inside. Sumps are often poorly illuminated. Inadequate lighting may create an accident situation.

Containment Cavities
These large below-grade areas are characterized by little or no air movement. Ventilation is always a problem. In addition, the possibility of oxygen deficiency exists. As well, welding and other gases may easily collect in these areas, creating toxic atmospheres. As these structures near completion, more confined spaces will exist as rooms are built off the existing structure.

Electrical Transformers
Electrical transformers are located on the jobsite. They often contain a nitrogen purge or dry air. Before they are opened, they must be well vented by having air pumped in. Workers, particularly electricians and power plant operators, will enter these transformers through hatches on top for various work-related reasons. Testing for oxygen deficiency and for toxic atmospheres is mandatory.

Heat Sinks
These larger pit areas hold cooling water in the event that there is a problem with the pumps located at the water supply to the plant--normally a river or lake--which would prevent cooling water from reaching the reactor core.

When in the pits, workers are exposed to welding fumes and electrical hazards, particularly because water accumulates in the bottom of the sink.

Generally, it is difficult to communicate with workers in the heat sink, because the rebar in the walls of the structure deaden radio signals.

Unusual Conditions

Confined Space within a Confined Space
By the very nature of construction, situations are created which illustrate one of the most hazardous confined spaces of all--a confined space within a confined space.

This situation appears as tanks within pits, pipe assemblies or vessels within pits, etc. In this situation, not only do the potential hazards associated with the outer confined space require testing, monitoring, and control, but those of the inner space also require similar procedures.

Often, only the outer space is evaluated. When workers enter the inner space, they are faced with potentially hazardous conditions. A good example of a confined space within a confined space is a vessel with a nitrogen purge inside a filtering water access pit. Workers entering the pit and/or the vessel should do so only after both spaces have been evaluated and proper control measures established.

Hazards in One Space Entering another Space
During an examination of confined spaces in construction, one often encounters situations which are not always easy to evaluate or control. For instance, a room or area which classifies as a confined space may be relatively safe for work.

However, access passages from other areas outside or adjacent to the room could, at some point, allow the transfer of hazardous agents into the "***safe***" one. One such instance would be a pipe coming through a wall into a containment room.

Welding fumes and other toxic materials generated in one room may easily travel through the pipe into another area, causing it to change from a safe to an unsafe workplace. A serious problem with a situation such as this is that workers working in the "**safe**" area are not aware of the hazards leaking into their area. Thus, they are not prepared to take action to avoid or control it.

Session Conclusion
In this discussion, we have defined inherent and induced hazards in confined spaces. We have examined typical confined spaces on construction sites and we have described representative hazards within these confined spaces.

Permitted Confined Space Entry Program
Definition of Confined Spaces Requiring an Entry Permit
Confined space:
- ✓ Is large enough or so configured that an employee can bodily enter and perform work.
- ✓ Has limited or restricted means for entry or exit (i.e. tanks, vessels, silos, storage bins, hoppers, vaults, and pits are spaces that may have limited means of entry).
- ✓ Is not designed for continuous employee occupancy.

Purpose
The Permit Required Space (**PRCS**) Program is provided to protect authorized employees that will enter confined spaces and may be exposed to hazardous atmospheres, engulfment in materials, conditions which may trap or asphyxiate due to converging or sloping walls, or contains any other safety or health hazards.

Many workplaces contain confined spaces not designed for human occupancy which due to their configuration hinder employee activities including entry, work and exit. Asphyxiation is the leading cause of death in confined spaces.

Subpart P applies to all open excavations in the earth's surface.
- ✓ All trenches are excavations.
- ✓ All excavations are not trenches.

Permit Required Confined Space Entry General Rules
During all confined space entries, the following safety rules must be strictly enforced:
1. Only authorized and trained employees may enter a confined space or act as safety watchmen/attendants.

2. No smoking is permitted in a confined space or near entrance/exit area.

3. During confined space entries, a watchmen or attendant must be present at all times.

4. Constant visual or voice communication will be maintained between the safety watchmen and employees entering a confined space.

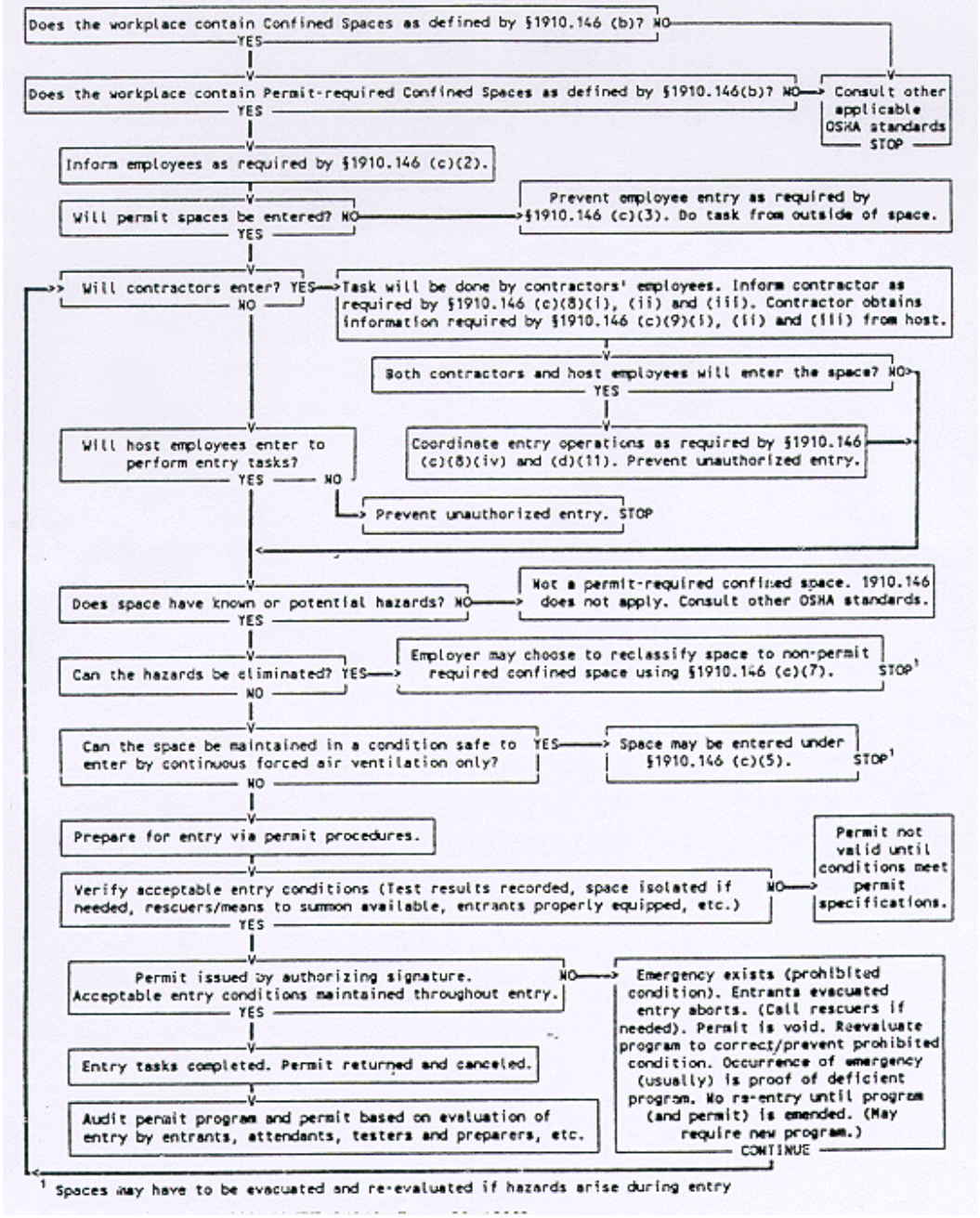
5. No bottom or side entry will be made or work conducted below the level any hanging material or material which could cause engulfment.

6. Air and oxygen monitoring is required before entering any permit-required confined space. Oxygen levels in a confined space must be between 19.5 and 23.5 percent. Levels above or below will require the use of an SCBA or other approved air supplied respirator. Additional ventilation and oxygen level monitoring is required when welding is performed. The monitoring will check oxygen levels, explosive gas levels and carbon monoxide levels. Entry will not be permitted if explosive gas is detected above one-half the Lower Explosive Limit (**LEL**).

7. To prevent injuries to others, all openings to confined spaces will be protected by a barricade when covers are removed.

Appendix A to §1910.146
Permit-Required Confined Space Decision Flow Chart
Note: Appendices A through F serve to provide information and non-mandatory guidelines to assist employers and employees in complying with the appropriate requirements of this section.

APPENDIX A TO §1910.146—PERMIT-REQUIRED CONFINED SPACE DECISION FLOW CHART



[58 FR 4549, Jan. 14, 1993; 58 FR 34846, June 29, 1993; 63 FR 66039, Dec. 1, 1998]

Here is a small clip-on style multi-purpose gas meter. We tied a string to lower the meter in the confined space to get a gas reading before entering.

Confined Space Entry Permit *Example*

Date & Time Issued		Date & time Expires	
Space I.D.		Supervisor	
Equipment Affected		Task	
Standby Team			
Pre-Entry Atmospheric Checks	Time (am - pm)		
	Oxygen		
	Explosive (% LEL)		
	Toxic (PPM)		
	Testers Signature		

Pre-entry Fluid System Isolation	Yes	No
Pumps /lines blinded, blocked, disconnected		
Ventilation Source Established		
Mechanical Forced Air		
Natural Ventilation		
Post Ventilation Pre-Entry Atmospheric Checks		
Time		
Oxygen (%)		
Explosive (% LEL		
Toxic (PPM)		
Tester Signature		
Communication Procedures Established per specific Confined Space SOP		
Rescue Procedures established per specific Confined Space SOP		

Training Verification - for the following persons & space to be entered	YES	NO
All persons entering Confined Space		
All persons acting as Supervisor for the Entry		
All persons assigned backup positions		
All persons assigned to monitor access and interior activities		
All persons assigned to emergency rescue team		

Equipment on Scene	YES	NO	NA		YES	NO	NA
Gas Monitor				Life Line			
Safety Harness				Hoisting Equipment			
Fall Arrest Gear				Powered Comm Eq.			
SCBAs				Air Line Respirators			
Protective Clothing				Elect Gear Properly Rated			

Periodic Atmospheric Checks				
Time (am - pm)				
Oxygen				
Explosive (% LEL)				
Toxic (PPM)				

Testers Signature				

A review of the work authorized by this permit and the information contained on this Entry Permit. Written instructions and safety procedures have been received and are understood. Entry cannot be approved if any squares are marked in the "**No**" column. This permit is not valid unless all appropriate items are completed.

Permit Prepared By: (Supervisor) _____

Approved By: (Unit Supervisor) _____

This permit to be kept at job site.

Return job site copy to Safety Office following job completion.

Copies: Safety Office, Unit Supervisor, Job site

Confined Space Duties & Responsibilities
Examples of Assignments

Employees

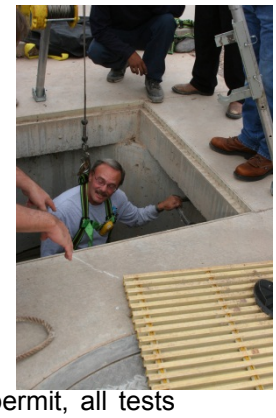
- Follow program requirements.
- Report any previously un-identified hazards associated with confined spaces.
- Do not enter any confined spaces that have not been evaluated for safety concerns.

Management

- Provide annual Confined Space training to all employees that may need confined space training.
- Ensure confined space assessments have been conducted.
- Annually review this program and all Entry Permits.

Rescue or Training Department

- Ensure proper training for entry & rescue teams.
- Provide proper equipment for entry & rescue teams.
- Ensure all permit required confined spaces are posted.
- Evaluate rescue teams and service to ensure they are adequately trained and prepared.
- Ensure rescue team at access during entry into spaces with Immediately Dangerous to Life or Health (IDLH) atmospheres.
- Provide annual confined space awareness training to all employees that may need confined space awareness training.



Entry Supervisor

Entry supervisors are responsible for the overall permit space entry and must coordinate all entry procedures, tests, permits, equipment and other relevant activities.

The following entry supervisor duties are required:

Know the hazards that may be faced during entry, including information on the mode, signs or symptoms, and consequences of the exposure.

Verify by checking that the appropriate entries have been made on the permit, all tests specified by the permit have been conducted, and that all procedures and equipment specified by the permit are in place before endorsing the permit and allowing entry to begin.

Terminate the entry and cancel the permit when the entry is complete or there is a need for terminating the permit.

Verify that rescue services are available and that the means for summoning them are operable.

Remove unauthorized persons who enter or attempt to enter the space during entry operations.

Determine whenever responsibility for a permit space entry operation is transferred and at intervals dictated by the hazards and operations performed within the space that entry operations remain consistent with the permit terms and that acceptable entry conditions are maintained.

Entry Attendants

At least one attendant is required outside the permit space into which entry is authorized for the duration of the entry operation. Responsibilities include:

- To know the hazards that may be faced during entry, including information on the mode, signs or symptoms, and consequences of the exposure.
- To be aware of possible behavioral effects of hazard exposure on entrants.
- To continuously maintain an accurate count of entrants in the permit space and ensures a means to accurately identify authorized entrants.
- To remain outside the permit space during entry operations until relieved by another attendant (once properly relieved, they may participate in other permit space activities, including rescue if they are properly trained and equipped).
- To communicate with entrants as necessary to monitor entrant status and alert entrants of the need to evacuate.
- To monitor activities inside and outside the space to determine if it is safe for entrants to remain in the space; orders the entrants to immediately evacuate if: the attendant detects a prohibited condition, detects entrant behavioral effects of hazard exposure, detects a situation outside the space that could endanger the entrants; or if the attendant cannot effectively and safely perform all the attendant duties.
- To summon rescue and other emergency services as soon as the attendant determines the entrants need assistance to escape the permit space hazards.
- To perform non-entry rescues as specified by that rescue procedure and entry supervisor and not to perform duties that might interfere with the attendants' primary duty to monitor and protect the entrants.

Entering a Confined Space

This space requires an emergency retrieval system, continuous air monitoring, and safety watch or two-way communication for safe entry.

Donning the personal protective equipment (**PPE**) necessary for confined space entry.

The full-body harness provides fully adjustable leg and shoulder straps for worker comfort and proper fit.

Stamped steel sliding back D-ring and subpelvic strap provide optimum force distribution.

Example of a "***D-Ring***" and fall protection harness used when entering a confined space. The D-Ring provides a compatible anchor point for connecting devices such as lanyards or retractable lifelines.

The shock absorbing lanyard provides a deceleration distance during a fall to reduce fall arrest forces for extra protection against injury.

Tripod-retrieval assembly in use for an entry into one of the many confined spaces.

Checking the cable tension and inertial locking mechanism of the retrieval assembly.

Correct use of this device prevents free-falls greater than 2 feet.

The entrant descends into the space as the attendant critiques the operation.

Dramatic rescue simulation using the tripod-retrieval system.

The entrant is now safely out of the space and is ready to return to his many other projects after this simulated exercise.

Duties of the Person Authorizing or in Charge of the Entry

The person who authorizes or is in charge of the permit entry confined space must comply with the following:

1. Make certain that all pre-entry requirements as outlined on the permit have been completed before any worker is allowed to enter the confined space.
2. Make certain that any required pre-entry conditions are present.
3. If an in-plant/facility rescue team is to be used in the event of an emergency, make sure they would be available. If your Employer does not maintain an in-plant rescue team, dial 911 on any telephone for the Rescue Squad.
4. Make sure that any communication equipment which would be used to summon either the in-plant rescue team or other emergency assistance is operating correctly.
5. Terminate the entry upon becoming aware of a condition or set of conditions whose hazard potential exceeds the limits authorized by the entry permit.

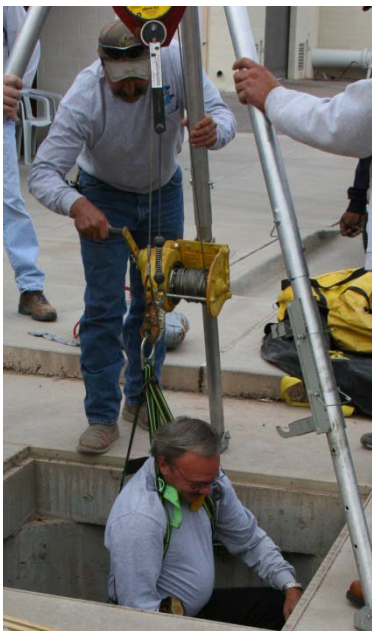
If the person who would otherwise issue an entry permit is in charge of the entry and present during the entire entry, then a written permit is not required if that person uses a checklist as provided in the section on "***Permits***".

This person may also serve as the attendant at the site.

Special Considerations During A Permit Required Entry
Certain work being performed in a permit entry confined space could cause the atmosphere in the space to change. Examples of this are welding, drilling, or sludge removal. In these situations, air monitoring of the confined space should be conducted on a continuous basis throughout the time of the entry.

If the workers leave the confined space for any significant period of time, such as for a lunch or other break, the atmosphere of the confined space must be retested before the workers reenter the confined space.

Unauthorized Persons
Take the following actions when unauthorized persons approach or enter a permit space while entry is under way:



1. Warn the unauthorized persons that they must stay away from the permit space,
2. Advise unauthorized persons that they must exit immediately if they have entered the space, and
3. Inform the authorized entrants and the entry supervisor if unauthorized persons have entered the permit space.

Entrants
All entrants must be authorized by the entry supervisor to enter permit spaces, have received the required training, have used the proper equipment, and observed the entry procedures and permit requirements.

The following entrant duties are required:
Know the hazards that may be faced during entry, including information on the mode, signs or symptoms, and consequences of the exposure;

Properly use the equipment required for safe entry;
Communicate with the attendant as necessary to enable the attendant to monitor the status of the entrants and to enable the attendant to alert the entrants of the need to evacuate the space if necessary;

Alert the attendant whenever; the entrant recognizes any warning signs or symptoms of exposure to a dangerous situation, or any prohibited condition is detected; and Exit the permit space as quickly as possible whenever the attendant or entry supervisor gives an order to evacuate the permit space, the entrant recognizes any warning signs or symptoms of exposure to a dangerous situation, the entrant detects a prohibited condition, or an evacuation alarm is activated.

Normal day for a pretreatment inspector. Get used to hydrogen sulfide gas because you will smell, and taste it the rest of your life.

Hazards

- ✓ **Explosive / Flammable Atmospheres**
- ✓ **Toxic Atmospheres**
- ✓ **Engulfment**
- ✓ **Asphyxiation**
- ✓ **Entrapment**
- ✓ **Slips & falls**
- ✓ **Chemical Exposure**
- ✓ **Electric Shock**
- ✓ **Thermal / Chemical Burns**
- ✓ **Noise & Vibration**

Hazard Control

Engineering Controls

- Locked entry points
- Temporary ventilation
- Temporary Lighting

Administrative Controls

- Signs
- Employee training
- Entry procedures
- Atmospheric Monitoring
- Rescue procedures
- Use of prescribed Personal Protective Equipment

Entry Standard Operating Procedures

This program outlines:

- Hazards
- Hazard Control & Abatement
- Acceptable Entry Conditions
- Means of Entry
- Entry Equipment Required
- Emergency Procedures

Permit Required Confined Space Entry General Rules

During all confined space entries, the following safety rules must be strictly enforced:

1. Only authorized and trained employees may enter a confined space or act as safety watchman/attendant.

2. No smoking is permitted in a confined space or near entrance/exit area.

3. During confined space entries, a watchman must be present at all times.

4. Constant visual or voice communication will be maintained between the safety watchman/attendant and employees entering a confined space.

5. No bottom or side entry will be made or work conducted below the level of any hanging material or material which could cause engulfment.

6. Air and oxygen monitoring is required before entering any permit-required confined space. Oxygen levels in a confined space must be between 19.5 and 23.5 percent. Levels above or below will require the use of an SCBA or other approved air supplied respirator. Additional ventilation and oxygen level monitoring is required when welding is performed.

The monitoring will check oxygen levels, explosive gas levels and carbon monoxide levels. Entry will not be permitted if explosive gas is detected above one-half the Lower Explosive Limit (**LEL**), or 10% of a specific gas explosive limit.

7. To prevent injuries to others, all openings to confined spaces will be protected by a barricade when covers are removed.

Confined Space Entry Procedures

Each employee who enters or is involved in the entry must:

1. Understand the procedures for confined space entry
2. Know the Hazards of the specific space
3. Review the specific procedures for each entry
4. Understand how to use entry and rescue equipment

Confined Space Entry Permits

Confined Space Entry Permits must be completed before any employee enters a permit-required confined space. The permit must be completed and signed by an authorized member of management before entry.

Permits will expire before the completion of the shift or if any pre-entry conditions change. Permits will be maintained on file for 12 months.

Contractor Entry
All work by non-company employees that involves the entry into confined spaces will follow the procedures of this program. The information of this program and specific hazards of the confined spaces to be entered will be provided to contractor management prior to commencing entry or work.

Important Rescue Service Questions
What is the availability of the rescue service?

Is it unavailable at certain times of the day or in certain situations?

What is the likelihood that key personnel of the rescue service might be unavailable at times?

If the rescue service becomes unavailable while an entry is underway, does it have the capability of notifying the employer so that the employer can instruct the attendant to abort the entry immediately?

Confined Space Training

***Training for Confined Space Entry include*s:**
1. Duties of entry supervisor, entrant and attendants
2. Confined space entry permits
3. Hazards of confined spaces
4. Use of air monitoring equipment
5. First aid and CPR training
6. Emergency action & rescue procedures
7. Confined space entry & rescue equipment
8. Rescue training, including entry and removal from representative spaces

Confined Space Training and Education
OSHA's General Industry Regulation, §1910.146 Permit-required confined spaces, contains requirements for practices and procedures to protect employees in general industry from the hazards of entry into permit-required confined spaces. This regulation does not apply to construction.

OSHA's Construction Safety and Health Regulations Part 1926 do not contain a permit-required confined space regulation. Subpart C, §1926.21 Safety training and education specifies training for personnel who are required to enter confined spaces and defines a "***confined or enclosed space***." These requirements are shown below.

§1926.21 Safety training and education. (Partial)
(b)(6)(i) All employees required to enter into confined or enclosed spaces shall be instructed as to the nature of the hazards involved, the necessary precautions to be taken, and in the use of protective and emergency equipment required. The employer shall comply with any specific regulations that apply to work in dangerous or potentially dangerous areas.

(ii) For purposes of paragraph (b)(6)(i) of this section, "***confined or enclosed space***" means any space having a limited means of egress, which is subject to the accumulation of toxic or flammable contaminants or has an oxygen deficient atmosphere. Confined or enclosed spaces include, but are not limited to, storage tanks, process vessels, bins, boilers, ventilation or exhaust ducts, sewers, underground utility vaults, tunnels pipelines, and open top spaces more than 4 feet in depth such as pits, tubs, vaults, and vessels.

OSHA's Construction Regulations also contain requirements dealing with confined space hazards in underground construction (Subpart S), underground electric transmission and distribution work (§1926.956), excavations (Subpart P), and welding and cutting (Subpart J).

Further guidance may be obtained from American National Standard ANSI Z117.1-1989, Safety Requirements for Confined Spaces. This standard provides minimum safety requirements to be followed while entering, exiting and working in confined spaces at normal atmospheric pressure. This standard does not pertain to underground mining, tunneling, caisson work or other similar tasks that have established national consensus standards.

Your Employer is Responsible for Certain Training Requirements

These are as follows:
1. **GENERAL** As an employer, your employer must ensure that all workers who must enter a permit entry confined space in the course of their work are informed of appropriate procedures and controls for entry into such spaces. These workers must be made aware of the fact that an unauthorized entry could be fatal, and that their senses are unable to detect and evaluate the severity of atmospheric hazards.

2. **TRAINING FOR AUTHORIZED ENTRANTS** Your employer must ensure that all authorized entrants know the emergency action plan and have received training covering the following subjects prior to entering any permit entry confined space:

a**. Hazard Recognition**: Each worker must understand the nature of the hazard before entering and the need to perform appropriate testing to determine if it is safe to enter.

b. **Use of Personal Protective Equipment**: Each employee must be taught the proper use of all personal protective equipment required for entry or rescue, and the proper use of protective barriers and shields.

c. **Self-Rescue**: Each worker must be trained to get out of the confined space as rapidly as possible without help whenever an order to evacuate is given by the attendant, whenever an automatic evacuation alarm is activated, or whenever workers recognize the warning signs of exposure to substances that could be found in the confined space. They must also be made aware of the toxic effects or symptoms of exposure to hazardous materials he could encounter in the confined space. This includes anything that could be absorbed through the skin or which could be carried through the skin by any solvents that are used. They must be trained to relay an alarm to the attendant and to attempt self- rescue immediately upon becoming aware of these effects.

d. **Special Work Practices or Procedures**: Each worker must be trained in any modifications of normal work practices that are necessary for permit entry confined space work.

3. **TRAINING FOR PERSONS AUTHORIZING OR IN CHARGE OF ENTRY** In addition to other requirements already covered, the person authorizing or in charge of entry shall be trained to recognize the effects of exposure to hazards that could be in the confined space. They must also carry out all duties that the permit assigns to them.

Rescue practice training. In the photo above, the sand bag represents a fallen victim.

4. **TRAINING FOR ATTENDANT** Any worker functioning as an attendant at a permit entry confined space must be trained in the company's emergency action plan, the duties of the attendant, and in;

a. Proper use of the communications equipment furnished for communicating with authorized workers entering the confined space or for summoning emergency or rescue services.

b. Authorized procedures for summoning rescue or other emergency services.

c. Recognition of the unusual actions of a worker which could indicate that they could be experiencing a toxic reaction to contaminants that could be present in the space.

d. Any training for rescuers, if the attendant will function as a rescuer also.

e. Any training for workers who enter the confined space, if the permit specifies that the duty of the attendant will rotate among the workers authorized to enter the confined space.

CONFINED SPACE AUTHORIZED ENTRANT'S LOG EXAMPLE

CONFINED SPACE: **DATE:**
TIME:

ENTRANT'S NAME (PRINT)	TIME IN	TIME OUT

ENTRY Attendant:

ENTRY Supervisor Review:

What do you think? Is this a dangerous confined space?

Would you weld inside a large pipe all alone? I am sure he is paid well, but is he safe and sound?

Confined Space Entry Procedure

Space _____ Date Last Modified _____

Place check mark in all applicable areas

	Hazards			Personal Protective Equipment
	Explosive / Combustion Hazard			Air supplied Respirator
	Exposed Electrical Circuits			Air Purifying Respirator
	Unguarded Machine Parts			Welding Protection
	Atmospheric Hazard			Gloves
	Potential Atmospheric Hazard			Hard Hat
	Thermal Hazard			**Ventilation Requirements**
	Chemical Hazard			Continuous ___cuft/min Note*: See Ventilation Guidelines for Confined Spaces for typical ventilation configurations and formulas.*
	Fall Hazard			
	Engulfment hazard		Note: *Additional ventilation may be required for hot work, grinding or other operations that would produce airborne fumes, mist or dust. Entry Supervisor must assess additional ventilation requirements base on tasks to be performed in the space*	
	Converging Walls			
	Floors slope-small cross-section			
	Slip Hazard			
	Entry Path			Vent Exhaust Point:
	Side entry			Vent Supply Point:
	Bottom entry			Space Volume
	Door			**Initial Purge Time=** 7.5 X _____(space volume) Effective Blower Capacity
	Top open entry			
	Top manhole entry			**20 Air Changes per Hour (ACH) for duration of entry**
	Hinged hatch			**Minimum initial Purge Time= 20 Minutes**

Entry & Rescue Equipment				Adequate Blower Capacity (ABC) = _____ ABC = Space Volume x 20 ACH 60 minutes
	Life Line			
	Floor level opening barrier			**Acceptable Entry Conditions**
	Body Harness			Confined Space Entry permit posted
	Tripod			Oxygen 19.5 23.5%
	Man Winch			Lower Explosive Level %
	Fall Arrest Unit			Toxic fumes/vapors Less than PEL
	Emerg Retrieval Line			No engulfing material in space
	Atmospheric Monitor			No hazardous chemicals or material
	Blower /Saddle / Trunks			Drained - Flushed
	Drop Light			Rescue Team Available on Site
	Communication Gear			Ventilation Established & Maintained
	Ladder			LOTO Electrical components in space
	Hand held radios			LOTO Mechanical Components in space
	Portable Lighting			LOTO All pipes to and from space

Other Hazards

Flammable Atmospheres
A flammable atmosphere generally arises from enriched oxygen atmospheres, vaporization of flammable liquids, byproducts of work, chemical reactions, concentrations of combustible dusts, and desorption of chemical from inner surfaces of the confined space.
An atmosphere becomes flammable when the ratio of oxygen to combustible material in the air is neither too rich nor too lean for combustion to occur. Combustible gases or vapors will accumulate when there is inadequate ventilation in areas such as a confined space.

Flammable gases such as acetylene, butane, propane, hydrogen, methane, natural or manufactured gases or vapors from liquid hydrocarbons can be trapped in confined spaces, and since many gases are heavier than air, they will seek lower levels as in pits, sewers, and various types of storage tanks and vessels. In a closed top tank, it should also be noted that lighter than air gases may rise and develop a flammable concentration if trapped above the opening.

The byproducts of work procedures can generate flammable or explosive conditions within a confined space. Specific kinds of work such as spray painting can result in the release of explosive gases or vapors. Welding in a confined space is a major cause of explosions in areas that contain combustible gas.

Chemical reactions forming flammable atmospheres occur when surfaces are initially exposed to the atmosphere, or when chemicals combine to form flammable gases. This condition arises when dilute sulfuric acid reacts with iron to form hydrogen or when calcium carbide makes contact with water to form acetylene.

Other examples of spontaneous chemical reactions that may produce explosions from small amounts of unstable compounds are acetylene-metal compounds, peroxides, and nitrates. In a dry state, these compounds have the potential to explode upon percussion or exposure to increased temperature.

Another class of chemical reactions that form flammable atmospheres arise from deposits of pyrophoric substances (carbon, ferrous oxide, ferrous sulfate, iron, etc.) that can be found in tanks used by the chemical and petroleum industry. These tanks containing flammable deposits will spontaneously ignite upon exposure to air.
Combustible dust concentrations are usually found during the process of loading, unloading, and conveying grain products, nitrated fertilizers, finely ground chemical products, and any other combustible material.

High charges of static electricity, which rapidly accumulate during periods of relatively low humidity (below 50%) can cause certain substances to accumulate electrostatic charges of sufficient energy to produce sparks and ignite a flammable atmosphere. These sparks may also cause explosions when the right air or oxygen to dust or gas mixture is present.

Toxic Atmospheres
The substances to be regarded as toxic in a confined space can cover the entire spectrum of gases, vapors, and finely-divided airborne dust in industry. The sources of toxic atmospheres encountered may arise from the following:

1. The manufacturing process (for example, in producing polyvinyl chloride, hydrogen chloride is used as well as vinyl chloride monomer, which is carcinogenic).
2. The product stored [removing decomposed organic material from a tank can liberate toxic substances, such as hydrogen sulfide (**H_2S**)].
3. The operation performed in the confined space (for example, welding or brazing with metals capable of producing toxic fumes).

During loading, unloading, formulation, and production, mechanical and/or human error may also produce toxic gases which are not part of the planned operation.

Carbon monoxide (**CO**) is a hazardous gas that may build up in a confined space. This odorless, colorless gas that has approximately the same density as air is formed from incomplete combustion of organic materials such as wood, coal, gas, oil, and gasoline; it can be formed from microbial decomposition of organic matter in sewers, silos, and fermentation tanks.

CO is an insidious toxic gas because of its poor warning properties. Early stages of CO intoxication are nausea and headache. CO may be fatal at as little as 1000 ppm or 10% in air, and is considered dangerous at 200 ppm or 2%, because it forms Carboxyhemoglobin in the blood which prevents the distribution of oxygen in the body.

CO is a relatively abundant colorless, odorless gas. Therefore, any untested atmosphere must be suspect. It must also be noted that a safe reading on a combustible gas indicator does not ensure that CO is not present. CO must be tested for specifically. The formation of CO may result from chemical reactions or work activities, therefore fatalities due to CO poisoning are not confined to any particular industry. There have been fatal accidents in sewage treatment plants due to decomposition products and lack of ventilation in confined spaces.

Another area where CO results as a product of decomposition is in the formation of silo gas in grain storage elevators. In another area, the paint industry, varnish is manufactured by introducing the various ingredients into a kettle, and heating them in an inert atmosphere, usually town gas, which is a mixture of carbon dioxide and nitrogen.

In welding operations, oxides of nitrogen and ozone are gases of major toxicologic importance, and incomplete oxidation may occur and carbon monoxide can form as a byproduct. Another poor work practice, which has led to fatalities, is the recirculation of diesel exhaust emissions. Increased CO levels can be prevented by strict control of the ventilation and the use of catalytic converters.

Procedures for Atmospheric Testing. - 1910.146 App B

OSHA Requirement

Subpart Title: General Environmental Controls

Atmospheric testing is required for two distinct purposes:
evaluation of the hazards of the permit space and verification that acceptable entry conditions for entry into that space exist.

(1) Evaluation testing. The atmosphere of a confined space should be analyzed using equipment of sufficient sensitivity and specificity to identify and evaluate any hazardous atmospheres that may exist or arise, so that appropriate permit entry procedures can be developed and acceptable entry conditions stipulated for that space.

Evaluation and interpretation of these data, and development of the entry procedure, should be done by, or reviewed by, a technically qualified professional (e.g., OSHA consultation service, or certified industrial hygienist, registered safety engineer, certified safety professional, certified marine chemist, etc.) based on evaluation of all serious hazards.

(2) Verification testing. The atmosphere of a permit space which may contain a hazardous atmosphere should be tested for residues of all contaminants identified by evaluation testing using permit specified equipment to determine that residual concentrations at the time of testing and entry are within the range of acceptable entry conditions. Results of testing (i.e., actual concentration, etc.) should be recorded on the permit in the space provided adjacent to the stipulated acceptable entry condition.

(3) Duration of testing. Measurement of values for each atmospheric parameter should be made for at least the minimum response time of the test instrument specified by the manufacturer.

(4) Testing stratified atmospheres. When monitoring for entries involving a descent into atmospheres that may be stratified, the atmospheric envelope should be tested a distance of approximately 4 feet (1.22 m) in the direction of travel and to each side. If a sampling probe is used, the entrant's rate of progress should be slowed to accommodate the sampling speed and detector response.

(5) Order of testing. A test for oxygen is performed first because most combustible gas meters are oxygen dependent and will not provide reliable readings in an oxygen deficient atmosphere.

Combustible gases are tested for next because the threat of fire or explosion is both more immediate and more life threatening, in most cases, than exposure to toxic gases and vapors. If tests for toxic gases and vapors are necessary, they are performed last.

This is a ten-minute escape air pack or emergency air supply. The plastic bag with go over your head during an emergency and provide enough air to get out of the hole. There are smaller versions of this system.

Confined Space Program *Multi-gas Meter Instructions*

Functional Buttons:

On/Off	Press black button and hold until display tells you to RELEASE. Turn on in a clean-air environment.
Mode	Press "mode" button at display prompt.
E Button	Press (E) button at display prompt.
Alarm Mode	Red lights flash and unit beeps. Beeps are more frequent at higher contaminant levels, or lower oxygen level.

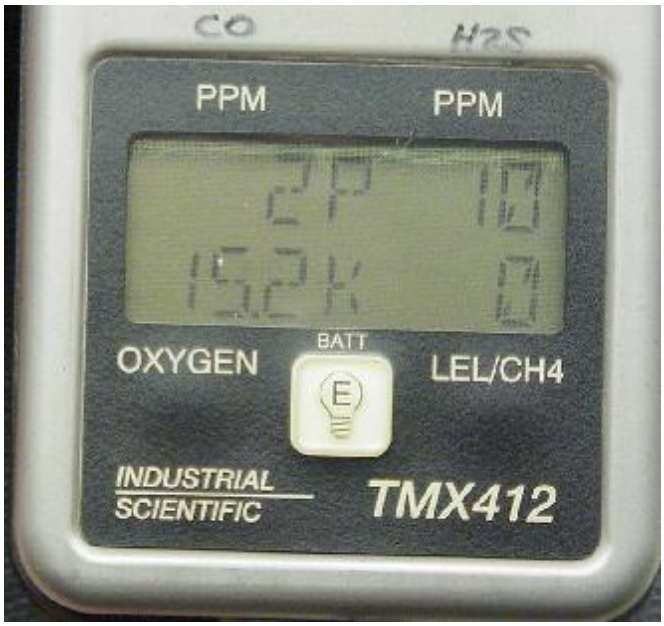
Forced air ventilation with a disposable air shaft.

Typical Display of the TMX412

Location of gases on display.

Example of a clean air display. Carbon monoxide (**CO**) and hydrogen sulfide (**H_2S**) are in ppm; oxygen (**O_2**) and lower explosive limit (**LEL**) readings are percentage values. The battery-life indicator is just right of the oxygen display (i.e., 20.9); each line represents about one hour of service remaining.

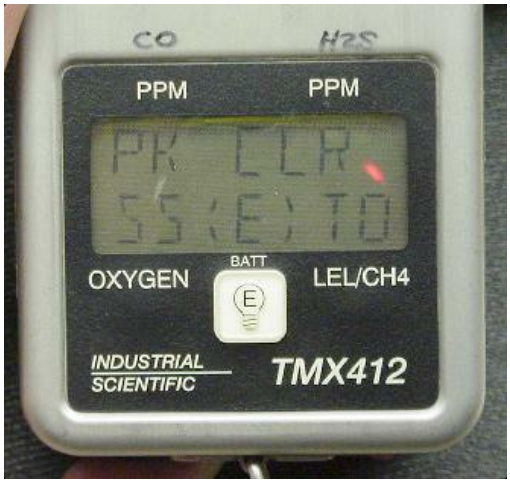
Peak Display Function



Example Display for Peak Mode: The display reads 2 ppm peak value for CO and 10 ppm peak value for H_2S (top line); 15.2 % for oxygen and 0 % for LEL (bottom line).

- Use the PEAK function to display highest recorded readings for CO, H2S, and LEL, and the lowest reading for O2.
- Readings are not erased when you turn the unit off. You must use the PEAK CLEAR function to erase the memory.
- Make sure you check the peak readings have been cleared before you start your monitoring session.
- Press mode button until display reads "P" (top line), and "K" (bottom line) (see photo).

Peak Clear Function



- Use the **PEAK CLEAR** function to clear peak readings from the internal memory. Readings are not erased when you turn the unit off. You must use the **PEAK CLEAR** function to erase the memory.
- Press mode button until display reads "**PK CLR PRESS (E) TO RESET**". After you press the (E) button, press mode button again until peak reading appears. Unit should now read 0,0 (top line), and 21, 0 (bottom line) assuming this was performed in a clean-air environment.

Zero Function and Calibration Function:

- Zero and Calibration Functions are performed by Attendant or as specified by the Supervisor or manufacturer.
- Special equipment and experience is necessary to properly perform these functions.

Documentation and Training:

- Make sure you are familiar with all of our confined space entry equipment, including the multi-gas monitor, before use.
- Make sure to document your air monitoring data (e.g., peak values and other relevant data) on the Confined Space Air Monitoring Data Form.

You need continued atmospheric monitoring during the entry in any confined space. Most entrants will carry two gas monitors for increased safety.

Atmospheric Testing Policy *Example*

Before entry, it is necessary to test the atmosphere in the confined space for oxygen levels, flammability, and/or any contaminants that have a potential to be present in that confined space. This testing must be done by a qualified person using equipment which has been approved for use in such areas.

The testing equipment itself should be checked to make sure it is working properly before using it. Follow the manufacturer's recommended procedures.

Testing of the confined spaces should be conducted throughout the entire portion of the space that workers will occupy during the entry. This testing shall be done without the use of ventilation systems.

Where the entry is vertical into the confined space, it is recommended that remote probes be used to measure the atmosphere at various levels. This is necessary because some gases and vapors are lighter or heavier than air and can accumulate at different levels in the confined space. Test outside the confined space to make sure the surrounding air is not contaminated.
Atmospheric conditions are considered unacceptable if oxygen levels are less than 19.5% or greater than 23.5%. Regulations define the following unacceptable levels of other hazards monitored:

1. A flammable gas, vapor or mist greater than 10% of its lower flammable limit (**LFL**). LFL means the minimum concentration of the flammable material which will ignite if an ignition source is present.
2. An airborne combustible dust at a concentration that obscures vision at a distance of five feet or less.
3. An atmospheric concentration of a substance greater than the allowed limit in the Material Safety Data Sheet for that substance.

If test results conclude that the atmospheric condition of the confined space is unacceptable, entry is prohibited until such conditions are brought into acceptable limits. This may be done by purging, cleaning and/or ventilating the space. Purging refers to the method by which gases, vapors, or other airborne impurities are displaced from a confined space.

The confined space may also be made non-flammable, non-explosive or otherwise chemically non-reactive by displacing or diluting the original atmosphere with steam or gas that is non-reactive with respect to that space, a process referred to as "***inerting***".

Irritant (Corrosive) Atmospheres

Irritant or corrosive atmospheres can be divided into primary and secondary groups. The primary irritants exert no systemic toxic effects (effects on the entire body).

Examples of primary irritants are chlorine, ozone, hydrochloric acid, hydrofluoric acid, sulfuric acid, nitrogen dioxide, ammonia, and sulfur dioxide. A secondary irritant is one that may produce systemic toxic effects in addition to surface irritation. Examples of secondary irritants include benzene, carbon tetrachloride, ethyl chloride, trichloroethane, trichloroethylene, and chloropropene.

Irritant gases vary widely among all areas of industrial activity. They can be found in plastics plants, chemical plants, the petroleum industry, tanneries, refrigeration industries, paint manufacturing, and mining operations. Prolonged exposure at irritant or corrosive concentrations in a confined space may produce little or no evidence of irritation. This may result in a general weakening of the defense reflexes from changes in sensitivity. The danger in this situation is that the worker is usually not aware of any increase in his/her exposure to toxic substances.

Asphyxiating Atmospheres
The normal atmosphere is composed approximately of 20.9% oxygen and 78.1% nitrogen, and 1% argon with small amounts of various other gases. Reduction of oxygen in a confined space may be the result of either consumption or displacement.

The consumption of oxygen takes place during combustion of flammable substances, as in welding, heating, cutting, and brazing. A more subtle consumption of oxygen occurs during bacterial action, as in the fermentation process.

Oxygen may also be consumed during chemical reactions as in the formation of rust on the exposed surface of the confined space (iron oxide). The number of people working in a confined space and the amount of their physical activity will also influence the oxygen consumption rate. A second factor in oxygen deficiency is displacement by another gas. Examples of gases that are used to displace air, and therefore reduce the oxygen level are helium, argon, and nitrogen.

Carbon dioxide may also be used to displace air and can occur naturally in sewers, storage bins, wells, tunnels, wine vats, and grain elevators. Aside from the natural development of these gases, or their use in the chemical process, certain gases are also used as inerting agents to displace flammable substances and retard pyrophoric reactions.

Gases such as nitrogen, argon, helium, and carbon dioxide, are frequently referred to as non-toxic inert gases but have claimed many lives. The use of nitrogen to inert a confined space has claimed more lives than carbon dioxide. The total displacement of oxygen by nitrogen will cause immediate collapse and death.

Carbon Dioxide
Carbon dioxide and argon, with specific gravities greater than air, may lie in a tank or manhole for hours or days after opening. Since these gases are colorless and odorless, they pose an immediate hazard to health unless appropriate oxygen measurements and ventilation are adequately carried out.

Oxygen Deprivation
Oxygen deprivation is one form of asphyxiation. While it is desirable to maintain the atmospheric oxygen level at 21% by volume, the body can tolerate deviation from this ideal. When the oxygen level falls to 17%, the first sign of hypoxia is deterioration to night vision, which is not noticeable until a normal oxygen concentration is restored. Physiologic effects are increased breathing volume and accelerated heartbeat.

Between 14-16% physiologic effects are increased breathing volume, accelerated heartbeat, very poor muscular coordination, rapid fatigue, and intermittent respiration. Between 6-10% the effects are nausea, vomiting, inability to perform, and unconsciousness. Less than 6%, the effects are spasmodic breathing, convulsive movements, and death in minutes.

Mechanical Hazards
If activation of electrical or mechanical equipment would cause injury, each piece of equipment should be manually isolated to prevent inadvertent activation before workers enter or while they work in a confined space. The interplay of hazards associated with a confined space, such as the potential of flammable vapors or gases being present, and the build-up of static charge due to mechanical cleaning, such as abrasive blasting, all influence the precautions which must be taken.

To prevent vapor leaks, flashbacks, and other hazards, workers should completely isolate the space. To completely isolate a confined space, the closing of valves is not sufficient. All pipes must be physically disconnected or isolation blanks bolted in place. Other special precautions must be taken in cases where flammable liquids or vapors may re-contaminate the confined space.

The pipes blanked or disconnected should be inspected and tested for leakage to check the effectiveness of the procedure. Other areas of concern are steam valves, pressure lines, and chemical transfer pipes. A less apparent hazard is the space referred to as a void, such as double walled vessels, which must be given special consideration in blanking off and inerting.

Thermal Effects
Four factors influence the interchange of heat between people and their environment. They are: (1) air temperature, (2) air velocity, (3) moisture contained in the air, and (4) radiant heat. Because of the nature and design of most confined spaces, moisture content and radiant heat are difficult to control.

As the body temperature rises progressively, workers will continue to function until the body temperature reaches approximately 102^{o}F.

When this body temperature is exceeded, the workers are less efficient, and are prone to heat exhaustion, heat cramps, or heat stroke. In a cold environment, certain physiologic mechanisms come into play, which tend to limit heat loss and increase heat production. The most severe strain in cold conditions is chilling of the extremities so that activity is restricted. Special precautions must be taken in cold environments to prevent frostbite, trench foot, and general hypothermia.

Protective Insulated Clothing
Protective insulated clothing for both hot and cold environments will add additional bulk to the worker and must be considered in allowing for movement in the confined space and exit time. Therefore, air temperature of the environment becomes an important consideration when evaluating working conditions in confined spaces.

Noise
Noise problems are usually intensified in confined spaces because the interior tends to cause sound to reverberate and thus expose the worker to higher sound levels than those found in an open environment.

This intensified noise increases the risk of hearing damage to workers, which could result in temporary or permanent loss of hearing. Noise in a confined space which may not be intense enough to cause hearing damage may still disrupt verbal communication with the emergency standby person on the exterior of the confined space. If the workers inside are not able to hear commands or danger signals due to excessive noise, the probability of severe accidents can increase.

Vibration
Whole body vibration may affect multiple body parts and organs, depending upon the vibration characteristics. Segmental vibration, unlike whole body vibration, appears to be more localized in creating injury to the fingers and hands of workers using tools, such as pneumatic hammers, rotary grinders or other hand tools which cause vibration.

Other Hazards
Some physical hazards cannot be eliminated because of the nature of the confined space or the work to be performed. These hazards include such items as scaffolding, surface residues, and structural hazards. The use of scaffolding in confined spaces has contributed too many accidents caused by workers or materials falling, improper use of guard rails, and lack of maintenance to insure worker safety.

The choice of material used for scaffolding depends upon the type of work to be performed, the calculated weight to be supported, and the surface on which the scaffolding is placed, as well as the substance previously stored in the confined space. Surface residues in confined spaces can increase the already hazardous conditions of electrical shock, reaction of incompatible materials, liberation of toxic substances, and bodily injury due to slips and falls. Without protective clothing, additional hazards to health may arise due to surface residues.

Structural hazards within a confined space such as baffles in horizontal tanks, trays in vertical towers, bends in tunnels, overhead structural members, or scaffolding installed for maintenance constitute physical hazards, which are exacerbated by the physical surroundings. In dealing with structural hazards, workers must review and enforce safety precautions to assure safety.

Abbreviations:
PEL - permissible exposure limit: Average concentration that must not be exceeded during 8-hour work shift of a 40-hour workweek.
STEL - Short-term exposure limit: 15-minute exposure limit that must not be exceeded during the workday.
REL - Recommended exposure limit: Average concentration limit recommended for up to a 10-hour workday during a 40-hour workweek.
IDLH - Immediately dangerous to life or health: Maximum concentration from which person could escape (in event of respirator failure) without permanent or escape-impairing effects within 30 minutes.

Required Confined Space Equipment Policy *Example*

Air Testing Equipment
All air-testing equipment should be calibrated in accordance with the manufacturer's instruction.

Oxygen Meters and Monitors
The oxygen content of the air in a confined space is the first and most important constituent to measure before entry is made. The acceptable range of oxygen is between 19.5 and 23.5 percent. This content is measured before flammability is tested because rich mixtures of flammable gases or vapors give erroneous measurement results.

For example, a mixture of 90 percent methane and 10 percent air will test nonflammable because there is not enough oxygen to support the combustion process in the flammability meters. This mixture will not support life and will soon become explosive if ventilation is provided to the space. Before entry, spaces must be ventilated until both oxygen content and flammability are acceptable.

Flammability Meters
Flammability meters are used to measure the amount of flammable vapors or gases in the atmosphere as a percent of the LEL/LFL. The oxygen content must be near 21 percent for results to be meaningful.

Toxic Air Contamination Testers
Tests for toxic contaminants must be specific for the target toxin. The instrument manufacturer should be consulted for interferences. Therefore, it is important to know the history of the confined space so proper tests can be performed. Part of hazard assessment is to identify all possible contaminants that could be in the confined space.

Protective Devices

Fall-Protection Equipment
Fall-protection equipment for confined spaces should be the chest-waist harness type to minimize injuries from uncontrolled movements when it arrests a worker's fall. This type of harness also permits easier retrieval from a confined space than a waist belt. Adjustable lanyards should be used to limit free fall to two feet before arrest.

Respirators
An industrial hygienist should select respirators on the basis of his or her evaluation of possible confined-space hazards. NIOSH-approved respirators should be identified in the approved procedure required by the confined-space entry permit. It is important to note that air-purifying respirators cannot be used in an oxygen deficient atmosphere.

Lockout/Tagout Devices
Lockout/tagout devices permit employees to work safely on de-energized equipment without fear that the devices will be accidentally removed. Lock and tag devices are required to withstand a 50-pound pull without failure. Devices used to block or restrain stored mechanical energy devices must be engineered for safety.

Safety Barriers
Safety barriers separate workers from hazards that cannot reasonably be eliminated by other engineering controls. Required barriers will be identified in the approved confined-space entry procedure.

Ground Fault Circuit Interrupters
Ground fault circuit interrupter must be used for all portable electrical tools and equipment in confined spaces because most workers will be in contact with grounded surroundings.

Emergency Response Equipment

Fire Extinguishers
"***Hot work***" inside a confined space requires that an approved fire extinguisher and a person trained in its use be stationed in the confined space or in a suitable vantage point where he or she could effectively suppress any fire that might result from the work.

First Aid Equipment
Blankets, first-aid kit, Stokes stretchers, and any other equipment that may be needed for first-response treatment must be available just outside the confined space. Medical and safety professionals should select equipment on the basis of their evaluations of the potential hazards in the confined space.

Retrieval Equipment
A tripod or another suitable anchorage, hoisting device, harnesses, wristlets, ropes, and any other equipment that may be needed to make a rescue must be identified in the confined-space safe-entry procedures.

It is important that this equipment be available for immediate use. Harnesses and retrieval ropes must be worn by entrants unless they would increase hazards to the entrants or impede their rescue.

Respiratory Protection Section

General
In the Respiratory Protection program, hazard assessment and selection of proper respiratory PPE is conducted in the same manner as for other types of PPE. In the control of those occupational diseases caused by breathing air contaminated with harmful dusts, fogs, fumes, mists, gases, smokes, sprays, or vapors, the primary objective shall be to prevent atmospheric contamination.

This shall be accomplished as far as feasible by accepted engineering control measures (for example, enclosure or confinement of the operation, general and local ventilation, and substitution of less toxic materials). When effective engineering controls are not feasible, or while they are being instituted, appropriate respirators shall be used.
References: OSHA Standards *Respiratory Protection* (**29 CFR 1910.134**)

Why Respirators Are Needed
Respirators protect against the inhalation of dangerous substances (vapors, fumes, dust, gases). They can also provide a separate air supply in a very hazardous situation.
Some of the health hazards that respirators prevent include
- *Lung damage*
- *Respiratory diseases*
- *Cancer and other illnesses.*

Respiratory Protection Responsibilities
The employer is responsible for:
- Providing training in the use and care of respirators.
- Ensuring that equipment is adequate, sanitary, and reliable.
- Allowing employees to leave area if ill, for breaks, and to obtain parts.
- Fit testing.
- Providing annual medical evaluations.
- Providing a powered air-purifying respirator (**PAPR**) if an employee cannot wear a tight-fitting respirator.

The employee is responsible for:
- Properly using respirators.
- Maintaining respirator properly.
- Reporting malfunctions.
- Reporting medical changes.

Selection of Respiratory Protection
When choosing the correct respiratory protection for your work environment, it is important to consider:
- Identification of the substance or substances for which respiratory protection is necessary
- A substance's material safety data sheet (**MSDS**) (it will state which type of respirator is most effective for the substance)
- Activities of the workers
- Hazards of each substance and its properties
- Maximum levels of air contamination expected
- Probability of oxygen deficiency
- Period of time workers will need to use the respiratory protection devices
- Capabilities and physical limitations of the device used

Types of Respirators The following is a description of different types of respirators.

Commonly Used Respirators (*Air Purifying*)

- **Disposable Dust** masks are worn over the nose and mouth to protect the respiratory system from certain nuisance dusts, mists, etc. They can only provide protection against particular contaminants as specified by the manufacturer (e.g., general dust, fiberglass, etc.). These dust masks cannot be fit tested, and are generally single use. They are not generally recognized as proper respiratory protection and may not be worn if a potential for overexposure exists. They are not included in most companies' Respiratory Protection Programs.
- **Half-Face Respirators** with interchangeable filter cartridges can protect the respiratory system from hazardous dusts, fumes, mists, etc. They can only provide protection against certain contaminants up to limited concentrations specified by the manufacturer for the particular cartridge type used (e.g., toluene, acetone). These generally operate under negative pressure within the respirator which is created by the wearer's breathing through the filter cartridges. As the protection is only gained if there is a proper seal of the respirator face piece, this type requires fit testing prior to respirator assignment and a fit check prior to each use.
- **Full-Face Respirators** operate under the same principle and requirements as the half-face type, however, they offer a better facepiece fit and also protect the wearer's eyes from particularly irritating gases or vapors.
- **Full-face, helmet or hood type powered air purifying respirators** (**PAPRs**) operate under positive pressure inside the facepiece using a battery operated motor blower assembly to force air through a filter cartridge into the wearer's breathing zone. Use of these respirators is also subject to the manufacturers' guidelines.

Less Commonly Used Types Respirators (Air Supplying)

- **Air-Line Respirators** supply clean air through a small diameter hose from a compressor or compressed air cylinders. The wearer must be attached to the hose at all times, which limits mobility. Use of these respirators is subject to the manufacturers' guidelines.
- **Self-Contained Breathing Apparatus** (**SCBA**) respirators supply clean air from a compressed air tank carried on the back of the wearer. These types of respirators are highly mobile and are used primarily for emergency response or rescue work, since only a limited amount of air can be supplied by a single tank, generally 20-60 minutes. Units must be thoroughly inspected on a monthly basis and written records must be kept of all inspections, operator training, etc. Use of these respirators is subject to the manufacturer's guidelines

Basic Types of Respirators

Air-purifying or filtering respirators. Such respirators are used when there is enough oxygen (at least 19.5 percent) and contaminants are present below IDLH level. The respirator filters out or chemically "**scrubs**" contaminants, usually with a replaceable filter. Use color-coded filter cartridges or canisters for different types of contaminants. It's important to select the right filter for the situation.

Air-supplying respirators. These respirators are required when air-purifying respirators aren't effective. Air-purifying respirators are not sufficient in the following settings:

- When there is not enough oxygen.
- Confined spaces.
- When contaminants cannot be filtered out.
- When contaminants are at or above IDLH level.

Different kinds of air-supplying respirators include

- Those connected by hose to stationary air supply (air line)
- Portable tank self-contained breathing apparatus (**SCBA**).

The Importance of Correct Fit

Even a tiny gap between the respirator and the face can allow contaminants to enter. Respirators should be comfortable and properly fitted. Proper fit includes:

- **Secure but not too tight**
- **No slipping or pinching**
- **Allowance for head movement and speech**

An OSHA-accepted qualitative fit test or quantitative fit test must be performed prior to an employee using any tight-fitting respirator. Tight-fitting respirators must be seal checked before each use by using positive- or negative-pressure check procedures or the manufacturer's instructions.

Respirator Filters/Cartridges

For protection against gases and vapors, the cartridges used for air-purifying respirators must be either equipped with an end-of-service-life indicator (**ESLI**), certified by NIOSH for the contaminant, or a cartridge change schedule has to be established.

For protection against particulates, there are nine classes of filters (three levels of filter efficiency, each with three categories of resistance to filter efficiency degradation). Levels of filter efficiency are 95 percent, 99 percent, and 99.97 percent. Categories of resistance to filter efficiency degradation are labeled N, R, and P.

Protection Factors

The protection factor of a respirator is an expression of performance based on the ratio of two concentrations: The contaminant concentration outside the respirator to the contaminant concentration inside the respirator.

Each class of respirator is also given an assigned protection factor (**APF**). The APF is a measure of the minimum anticipated level of respiratory protection that a properly functioning respirator or class of respirators would provide to a percentage of properly fitted and trained users.
When a contaminant concentration is known, the APF can be used to estimate the concentration inside a particular type of respirator worn by a user.

Who Cannot Wear a Respirator?

Respirator fit is essential. Employees must have a medical checkup to make sure they can wear respirators safely. Generally, respirators cannot be worn when a person:

- **Wears glasses or personal protective equipment that interferes with the seal of the face piece to the face of the user.**
- **Has facial hair that comes between the sealing surface of the face piece and the face or interferes with valve function.**
- **Has a breathing problem, such as asthma.**
- **Has a heart condition.**
- **Is heat sensitive.**

Sometimes a person's facial features will not permit a good fit. Check with the supervisor or medical department if the fit is a problem.

Checking for Damage
Before each use, make sure there are no holes, tears, etc., in the respirator. Rubber parts can wear out and should be checked very carefully every time a respirator is used. Replace worn and damaged parts when necessary. Make sure air and oxygen cylinders are fully charged.

Staying Prepared for Respirator Use
Respirators are bulky and awkward, so getting used to them takes practice. Possible problems with wearing respirators may include heat exhaustion or heat stroke. Be alert for symptoms, use the "**buddy system**," and wear a lifeline or harness when necessary. Drink plenty of fluids and take frequent breaks.

Poor maneuverability. Practice with respirators in narrow passages, on ladders, etc., if your use of respirators may be in these types of conditions.

Using up the air supply. When a SCBA is in use, keep checking the gauges and listening for alarms; be ready to leave the area immediately if there is a problem.

Panic. Remember the importance of staying calm in a hot, stressful, or awkward situation.

Cleaning Respirators
Respirators should be cleaned and disinfected after every use. Check the respirator for damage before putting it away; look for holes, cracks, deterioration, dented cartridges, etc. If any damage is found, it should be reported to a supervisor. Respirators stored for emergency use must be inspected monthly when not in use, as well as after each use. Respirators should be stored away from light, heat, cold, chemicals, and dust. Store respirators in a "***normal***" (natural, undistorted) position to hold their shape. Do not allow respirators to get crushed, folded, or twisted.

OSHA Overview

OSHA requires that supervisors consult with employees and encourage their participation in the process safety management plan. In fact, managers must have a written plan of action for employee participation in process safety management. Employee participation is critical because…

- **Employees know a lot about the process which they work upon**
- **They play key roles in making sure that process operation is conducted safely.**

Operating Procedures

Managers must furnish written operating procedures that clearly explain how to perform each covered process safely. The procedures must be accurate and must be written in language that people can understand. Avoid technical jargon and, if necessary, supply translations.

Operating procedures must include at least the following:

- Operating steps for initial startup, normal and temporary operations, emergency shutdown (including when it's called for and who does it), emergency operations, normal shutdown, and startup after a turnaround or an emergency shutdown
- Operating limits, including what happens if workers don't conform to operating limits and how to avoid or correct such problems
- Safety and health considerations, such as chemical or other hazards, precautions to prevent exposure, quality and inventory control for chemicals, and what to do if an employee is exposed to a hazardous substance
- Safety systems and their functions, including up-to-date operating procedures and safe work practices.

Contractor Employees

Process safety training and safety programs are also required for contractors who work on-site. Managers must check out the safety performance and programs of any contractors being considered for maintenance, repair, turnaround, major renovation, or specialty work on or around a process covered by the regulation.

When a contractor is hired, the manager must provide the contractor with information on the hazards of the process the contractor will work on. To further ensure contractor safety, managers must also

- ❖ provide the contractor with information on safe work practices for the process they're involved with and tell them what to do in an emergency
- ❖ keep a log of contractor employees' injuries or illnesses related to their work in process areas
- ❖ evaluate the contractor's performance to make sure they're living up to their safety obligations set by the standard.

The Contractor has Responsibilities, too

- Document that employees are trained to recognize hazards and to follow safe work practices on the job
- Make sure that the contractor's employees understand potential job-related hazards, are trained to work safely, and follow the safety rules of the facility in which they're working.

Written Respiratory Protection Program

This paragraph requires the employer to develop and implement a written respiratory protection program with required worksite-specific procedures and elements for required respirator use. The program must be administered by a suitably trained program administrator. In addition, certain program elements may be required for voluntary use to prevent potential hazards associated with the use of the respirator.

The Small Entity Compliance Guide contains criteria for the selection of a program administrator and a sample program that meets the requirements of this paragraph. Copies of the Small Entity Compliance Guide will be available on or about April 8, 1998 from the Occupational Safety and Health Administration's Office of Publications, Room N 3101, 200 Constitution Avenue, NW, Washington, DC, 20210 (202-219-4667).

(c)(1) In any workplace where respirators are necessary to protect the health of the employee or whenever respirators are required by the employer, the employer shall establish and implement a written respiratory protection program with worksite-specific procedures. The program shall be updated as necessary to reflect those changes in workplace conditions that affect respirator use. The employer shall include in the program the following provisions of this section, as applicable:

(c)(1)(i) Procedures for selecting respirators for use in the workplace;

(c)(1)(ii) Medical evaluations of employees required to use respirators;

(c)(1)(iii) Fit testing procedures for tight-fitting respirators;

(c)(1)(iv) Procedures for proper use of respirators in routine and reasonably foreseeable emergency situations;

(c)(1)(v) Procedures and schedules for cleaning, disinfecting, storing, inspecting, repairing, discarding, and otherwise maintaining respirators;

(c)(1)(vi) Procedures to ensure adequate air quality, quantity, and flow of breathing air for atmosphere-supplying respirators;

(c)(1)(vii) Training of employees in the respiratory hazards to which they are potentially exposed during routine and emergency situations;

Example of RP Employee Responsibilities

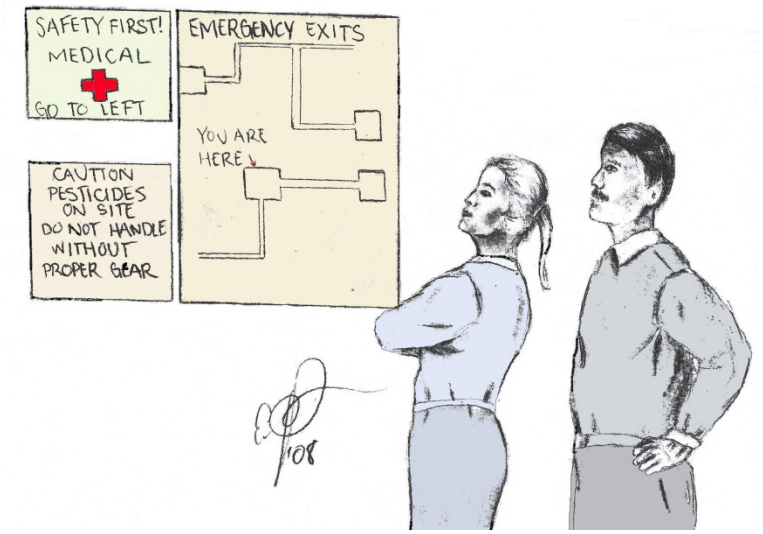
All Employees shall follow the requirements of the Respiratory Protection Program.

Management

- Implement the requirements of this program.
- Provide a selection of respirators as required.
- Enforce all provisions of this program.
- Appoint a ***Specific Designated*** individual to conduct the respiratory protection program.

Administrative Department

- Review sanitation/storage procedures.
- Ensure respirators are properly stored, inspected and maintained.
- Monitor compliance for this program.
- Provide training for affected Employees.
- Review compliance and ensure monthly inspection of all respirators.
- Provide respirator fit testing.



Designated-Occupational Health Care Provider

- Conducts medical aspects of program.

Program Administrator

Each Department will designate a program administrator who is qualified by appropriate training or experience that is commensurate with the complexity of the program to administer or oversee the respiratory protection program and conduct the required evaluations of program effectiveness.

Voluntary Use of Respirators is Prohibited

OSHA requires that voluntary use of respirators, when not required by the Employer, must be controlled as strictly as under required circumstances. To prevent violations of the Respiratory Protection Standard, Employees are not allowed voluntary use of their own or Employer supplied respirators of any type.

Exception: Employees whose only use of respirators involves the voluntary use of filtering (non-sealing) face pieces (dust masks).

See appendix D in the rear.

Respiratory Protection Program Statement *Example*

Facility _____
Policy Statement
A respiratory protection program is hereby established so as to coordinate the use and maintenance or respiratory protective equipment as determined necessary to:

1. Reduce Personnel exposure to toxic chemical agents, harmful dusts, mist and fumes and
2. Allow trained personnel to work safely in hazardous environments, such as welding, oxygen deficient atmospheres, toxic atmospheres, etc.

Designation of Program Administrator

Management has designated _____
to be responsible for the respiratory protection program at this facility. He/she has been delegated authority by Management to make decisions and implement changes in the respirator program anywhere in this facility.

The following responsibilities apply:

1. Supervision of respirator selection process and procedures
2. Establishment of respiratory protection training sessions
3. Establishment of a continuing program of cleaning and inspections
4. Establishment of medical screening program
5. Establishment of issuing procedures
6. Establishment of periodic inspections
7. Continuing evaluation of all aspects of the respiratory protection program to assure continued effectiveness
8. Establishment of annual fit tests procedures

Any questions or problems concerning respirators or their use should be directed to the Program Administrator

_____ _____
Facility Manager Date

Program Evaluation
Evaluations of the workplace are necessary to ensure that the written respiratory protection program is being properly implemented; this includes consulting with employees to ensure that they are using the respirators properly. Evaluations shall be conducted as necessary to ensure that the provisions of the current written program are being effectively implemented and that it continues to be effective.

Program evaluation will include discussions with employees required to use respirators to assess the employees' views on program effectiveness and to identify any problems.

Any problems that are identified during this assessment shall be corrected. Factors to be assessed include, but are not limited to:

- Respirator fit (including the ability to use the respirator without interfering with effective workplace performance);
- Appropriate respirator selection for the hazards to which the employee is exposed;
- Proper respirator use under the workplace conditions the employee encounters; and
- Proper respirator maintenance.

RP Recordkeeping

The employer will retain written information regarding medical evaluations, fit testing, and the respiratory protection program.

This information will facilitate employee involvement in the respiratory protection program, assist the Employer in auditing the adequacy of the program, and provide a record for compliance determinations by OSHA.

Training and Information
Effective training for employees who are required to use respirators is essential. The training must be comprehensive, understandable, and recur annually and more often if necessary. Training will be provided prior to requiring the employee to use a respirator in the workplace.

The training shall ensure that each employee can demonstrate knowledge of at least the following:
- Why the respirator is necessary and how improper fit, usage, or maintenance can compromise the protective effect of the respirator
- Limitations and capabilities of the respirator
- How to use the respirator effectively in emergency situations, including situations in which the respirator malfunctions
- How to inspect, put on and remove, use, and check the seals of the respirator
- Procedures for maintenance and storage of the respirator
- How to recognize medical signs and symptoms that may limit or prevent the effective use of respirators
- The general requirements of this program

Retraining shall be conducted annually and when:
- changes in the workplace or the type of respirator render previous training obsolete
- inadequacies in the employee's knowledge or use of the respirator indicate that the employee has not retained the requisite understanding or skill
- other situation arises in which retraining appears necessary to ensure safe respirator use

Training is divided into the following sections:

Classroom Instruction
1. Overview of the Employer’s Respiratory Protection Program & OSHA Standard.
2. Respiratory Protection Safety Procedures.
3. Respirator Selection.
4. Respirator Operation and Use.
5. Why the respirator is necessary.
6. How improper fit, usage, or maintenance can compromise the protective effect.
7. Limitations and capabilities of the respirator.
8. How to use the respirator effectively in emergency situations, including respirator malfunctions.
9. How to inspect, put on and remove, use, and check the seals of the respirator.
10. Procedures for maintenance and storage of the respirator.
11. How to recognize medical signs and symptoms that may limit or prevent the effective use of respirators.
12. Change out schedule and procedure for air purifying respirators.

Respiratory Protection Program

Training Certificate *Example*

Name:_____

Department:_____ Date:_____

I have received Training on the Respiratory Protection Program. The Training included the following:

Classroom Training

- ✓ Overview of the Company Respiratory Protection Program
- ✓ Respiratory Protection Safety Procedures
- ✓ Respirator Selection
- ✓ Respirator Operation and Use
- ✓ Why the respirator is necessary
- ✓ How improper fit, usage, or maintenance can compromise the protective effect.
- ✓ Limitations and capabilities of the respirator.
- ✓ How to use the respirator effectively in emergency situations, including respirator malfunctions
- ✓ How to inspect, put on and remove, use, and check the seals of the respirator.
- ✓ Procedures for maintenance and storage of the respirator.
- ✓ How to recognize medical signs and symptoms that may limit or prevent the effective use of respirators.
- ✓ Respirator filter & cartridge changeout schedule
- ✓ The general requirements of this program

Hands-on Training

- ✓ Respirator Inspection
- ✓ Respirator cleaning and sanitizing
- ✓ Fit Check
- ✓ Record Keeping
- ✓ Respirator Storage
- ✓ Emergencies

_____

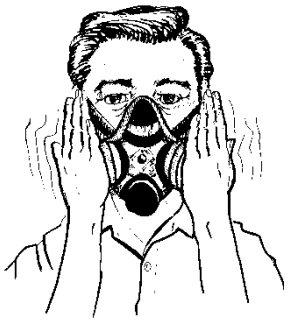
Employee Signature

_____

Trainer's Signature

Fit Testing Hands-On Respirator Training

(***see appendix A for more information***)



1. **Respirator Inspection**
2. **Respirator cleaning and sanitizing**
3. **Record Keeping**
4. **Respirator Storage**
5. **Respirator Fit Check**
6. **Emergencies**

Basic Respiratory Protection Safety Procedures

1. Only authorized and trained employees may use respirators. Those employees may use only the respirator that they have been trained on and properly fitted to use.
2. Only physically qualified employees may be trained and authorized to use respirators. A pre-authorization and annual certification by a qualified physician will be required and maintained. Any changes in an Employee's health or physical characteristics will be reported to the Occupational Health Department and will be evaluated by a qualified physician.
3. Only the proper prescribed respirator or SCBA may be used for the job or work environment. Air cleansing respirators may be worn in work environments when oxygen levels are between 19.5 percent to 23.5 percent and when the appropriate air cleansing canister, as determined by the Manufacturer and approved by NIOSH or MESA, for the known hazardous substance is used. SCBAs will be worn in oxygen deficient and oxygen rich environments (below 19.5 percent or above 23.5 percent oxygen).
4. Employees working in environments where a sudden release of a hazardous substance is likely will wear an appropriate respirator for that hazardous substance (example: employees working in an ammonia compressor room will have an ammonia APR respirator on their person.).
5. Only SCBAs will be used in oxygen deficient environments, environments with an unknown hazardous substance or unknown quantity of a known hazardous substance or any environment that is determined "***Immediately Dangerous to Life or Health***" (IDLH).
6. Employees with respirators loaned on "permanent check out" will be responsible for the sanitation, proper storage and security. Respirators damaged by normal wear will be repaired or replaced by the employer when returned.
7. The last employee using a respirator and/or SCBA that are available for general use will be responsible for proper storage and sanitation. Monthly and after each use, all respirators will be inspected with documentation to assure its availability for use.
8. All respirators will be located in a clean, convenient and sanitary location.
9. In the event that employees must enter a confined space, work in environments with hazardous substances that would be dangerous to life or health should an RPE fail (a SCBA is required in this environment), and/or conduct a HAZMAT entry, a "**buddy system**" detail will be used with a safety watchman with constant voice, visual or signal line communication. Employees will follow the established emergency response program and/or confined space entry program when applicable.
10. Management will establish and maintain surveillance of jobs and work place conditions and degree of employee exposure or stress to maintain the proper procedures and to provide the necessary RPE.
11. Management will establish and maintain safe operation procedures for the safe use of RPE with strict enforcement and disciplinary action for failure to follow all general and specific safety rules. Standard operation procedures for general RPE use will be maintained as an attachment to the respiratory protection program and standard operation procedures for RPE use under emergency response situations will be maintained as an attachment to the emergency response program.

Selection of Respirators

The employer is responsible for and needs to have evaluated the respiratory hazard(s) in each workplace, identified relevant workplace and user factors and have based respirator selection on these factors. Also included are estimates of employee exposures to respiratory hazard(s) and an identification of the contaminant's chemical state and physical form.

This selection has included appropriate protective respirators for use in IDLH atmospheres, and has limited the selection and use of air-purifying respirators. All selected respirators are NIOSH-certified.

Filter Classifications - These classifications are marked on the filter or filter package
N-Series: Not Oil Resistant

- Approved for non-oil particulate contaminants
- Examples: dust, fumes, mists not containing oil

R-Series: Oil Resistant

- Approved for all particulate contaminants, including those containing oil
- Examples: dusts, mists, fumes
- Time restriction of 8 hours when oils are present

P-Series: Oil Proof

- Approved for all particulate contaminants including those containing oil
- Examples: dust, fumes, mists
- See Manufacturer's time use restrictions on packaging

Respirators for IDLH Atmospheres

- The following respirators will be used in IDLH atmospheres:
- A full face piece pressure demand SCBA certified by NIOSH for a minimum service life of thirty minutes, or
- A combination full face piece pressure demand supplied-air respirator (**SAR**) with auxiliary self-contained air supply.
- Respirators provided only for escape from IDLH atmospheres shall be NIOSH-certified for escape from the atmosphere in which they will be used.

Respirators for Atmospheres that are not for IDLH

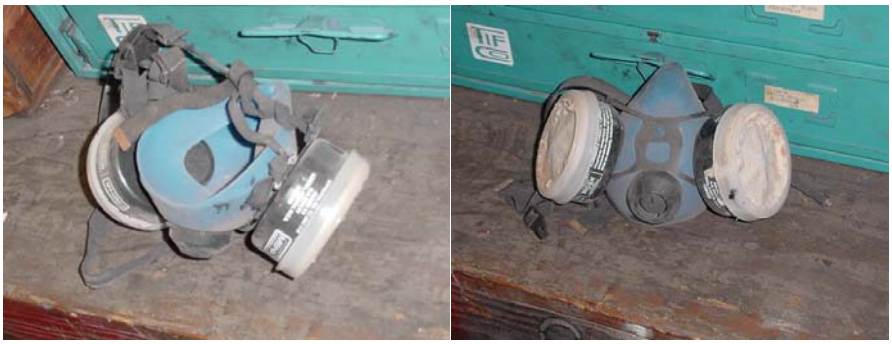
The respirators selected shall be adequate to protect the health of the employee and ensure compliance with all other OSHA statutory and regulatory requirements, under routine and reasonably foreseeable emergency situations. The respirator selected shall be appropriate for the chemical state and physical form of the contaminant.

Identification of Filters & Cartridges

All filters and cartridges shall be labeled and color coded with the NIOSH approval label; the label is not to be removed and must remain legible. A change out schedule for filters and canisters has been developed to ensure the elements of the respirators remain effective.

Respirator Filter & Canister Replacement

An important part of the Respiratory Protection Program includes identifying the useful life of canisters and filters used on air-purifying respirators. Each filter and canister shall be equipped with an end-of-service-life indicator (**ESLI**) certified by NIOSH for the contaminant; or If there is no ESLI appropriate for conditions a change schedule for canisters and cartridges that is based on objective information or data that will ensure that canisters and cartridges are changed before the end of their service life.



It is unacceptable maintenance and storage (OSHA Violation).

Filter & Cartridge Change Schedule
Stock of spare filters and cartridges shall be maintained to allow immediate change when required or desired by the employee.

Cartridges shall be changed based on the most limiting factor below:
- Prior to expiration date
- Manufacturer's recommendations for the specific use and environment
- After each use
- When requested by employee
- When contaminate odor is detected
- When restriction to air flow has occurred as evidenced by increased effort by user to breathe normally
- Cartridges shall remain in their original sealed packages until needed for immediate use

Filters shall be changed on the most limiting factor below:
- Prior to expiration date
- Manufacturer's recommendations for the specific use and environment
- When requested by employee
- When contaminate odor is detected
- When restriction to air flow has occurred as evidenced by increased effort by user to breathe normally
- When discoloring of the filter media is evident
- Filters shall remain in their original sealed package until needed for immediate use.

RESPIRATORY PROTECTION PROGRAM CHECKLIST			PAGE 1 OF 1 PAGES		
DIVISION:	**SECTION:**	**SUPERVISOR:**	**DATE:**		
			YES	NO	NA
1	Is respiratory protection (RP) being worn in the section?				
2	Has air sampling been accomplished that mandates using RP?				
3	Where air sampling results greater than Occupational Exposure Limits? (If NO, why are you using a respirator?)				
4	Has a Hazard Assessment been generated concerning the task or process that placed the section on the RP Program?				
5	Have all processes that may warrant the use of RP been evaluated? (If NO, request an assessment from the Department Safety Analyst /Personnel Safety, unless the operation is emergency response).				
6	Have workers received physicals and been found medically qualified to wear RP?				
7	Is there documentation that workers were formally briefed on air sampling results and why RP is required?				
8	Is respiratory protection training and fit-testing documentation available on everyone who wears a respirator?				
9	Are RP wearers being fit-tested at least annually?				
10	Are section employees wearing RP voluntarily when conditions have not mandated their use?				
11	Are employees wearing contacts in hazardous atmospheres or using eye-wear that negates face to face piece seal?				
12	Do RP users have facial hair that negates face to face piece seal?				
13	Has a respirator inventory been compiled that list the type of respirator(s) used in the workplace? (Use Respirator Inventory Worksheet attach to this checklist)				
14	Has the Section Supervisor received formal RP training on OSHA, City Personnel Safety and Respiratory Protection Program requirements and his or her responsibilities?				
15	Does the section have written standard operating instructions governing the selection, fit-testing, use, cleaning, storage and maintenance of respirators?				
16	Is the Fire Department the only source being used to charge SCBA's with compressed air?				
17	Are SCBA's being inspected at least every 30 days?				
18	Does the section have on hand, applicable OSHA, CITY, and Section Respiratory Protection Program guidance documents?				
19	Are periodic audits of the section's RP program conducted with discrepancies tracked until closed out?				
20	Have program deficiencies been elevated to the Director and Department Safety Analyst?				

SURVEYED BY:	REVIEWED BY:

Respiratory Protection Schedule by Job and Working Condition

The employer needs to maintain a Respiratory Protection Schedule by Job and working condition. This schedule is provided to each authorized and trained employee.

The Schedule provides the following information:

1. Job/Working conditions.
2. Work location.
3. Hazards present.
4. Type of respirator or SCBA required.
5. Type of filter/canister required.
6. Location of respirator or SCBA.
7. Filter/Cartridge change out schedule.

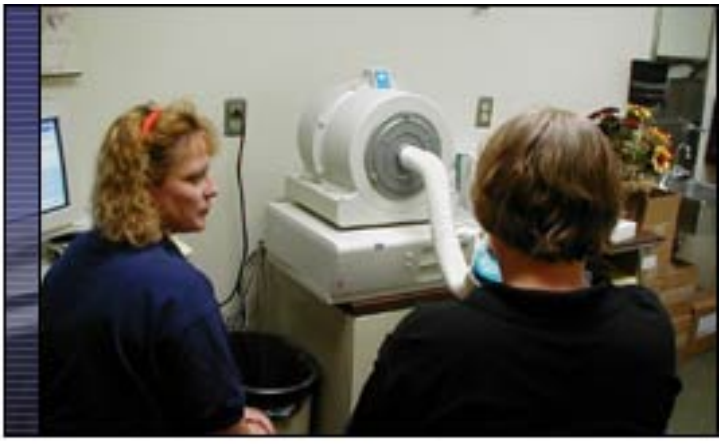
The schedule will be reviewed and updated at least annually and whenever any changes are made in the work environments, machinery, equipment, or processes or if respirator different respirator models are introduced or existing models are removed.

Permanent respirator Schedule Assignments are:
Each person who engages in welding will have their own employer provided dust-mist-fume filter APR. This respirator will be worn during all welding operations.

Physical and Medical Qualifications
Records of medical evaluations must be retained and made available in accordance with 29 CFR 1910.1020.

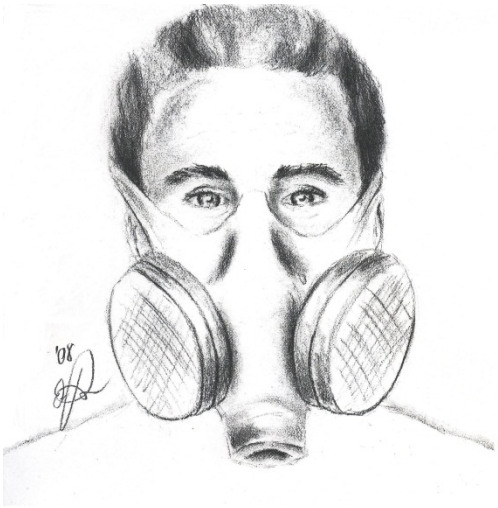
Medical Evaluation Required
Using a respirator may place a physiological burden on employees that varies with the type of respirator worn, the job and workplace conditions in which the respirator is used, and the medical status of the employee. The Employer is required to provide a medical evaluation to determine the employee's ability to use a respirator before the employee is fit tested or required to use the respirator in the workplace.

Medical Evaluation Procedures
The employee will be provided a medical questionnaire by the designated Occupational Health Care Provider.



Follow-up Medical Examination

The employer shall ensure that a follow-up medical examination is provided for an employee who gives a positive response to any question among questions in Part B of the questionnaire or whose initial medical examination demonstrates the need for a follow-up medical examination. The follow-up medical examination shall include any medical tests, consultations, or diagnostic procedures that the physician deems necessary to make a final determination.



Administration of the Medical Questionnaire and Examinations.
The medical questionnaire and examinations shall be administered confidentially during the employee's normal working hours or at a time and place convenient to the employee. The medical questionnaire shall be administered in a manner that ensures that the employee understands its content. The employer shall provide the employee with an opportunity to discuss the questionnaire and examination results with the Physician.

Supplemental Information for the Physician
The following information must be provided to the physician before the Physician makes a recommendation concerning an employee's ability to use a respirator.

- The type and weight of the respirator to be used by the employee
- The duration and frequency of respirator use (including use for rescue and escape)
- The expected physical work effort
- Additional protective clothing and equipment to be worn
- Temperature and humidity extremes that may be encountered
- Any supplemental information provided previously to the physician regarding an employee need not be provided for a subsequent medical evaluation if the information and the physician remain the same.

The employer has provided the physician with a copy of the written respiratory protection program and a copy of the OSHA Standard 1910.134

Acronyms
Qualitative fit test (QLFT) means a pass/fail fit test to assess the adequacy of respirator fit that relies on the individual's response to the test agent.

Quantitative fit test (QNFT) means an assessment of the adequacy of respirator fit by numerically measuring the amount of leakage into the respirator.

Medical Determination
In determining the employee's ability to use a respirator, the employer shall:

- Obtain a written recommendation regarding the employee's ability to use the respirator from the physician. The recommendation shall provide only the following information:
  - Any limitations on respirator use related to the medical condition of the employee, or relating to the workplace conditions in which the respirator will be used, including whether or not the employee is medically able to use the respirator.
  - The need, if any, for follow-up medical evaluations.
  - A statement that the Physician has provided the employee with a copy of the physician's written recommendation.

- If the respirator is a negative pressure respirator and the physician finds a medical condition that may place the employee's health at increased risk if the respirator is used, the employer shall provide an APR if the physician's medical evaluation finds that the employee can use such a respirator; if a subsequent medical evaluation finds that the employee is medically able to use a negative pressure respirator, then the employer is no longer required to provide an APR.

Additional Medical Evaluations
At a minimum, the employer shall provide additional medical evaluations that comply with the requirements of this section if:
- An employee reports medical signs or symptoms that are related to the ability to use a respirator
- A physician, supervisor, or the respirator program administrator informs the employer that an employee needs to be reevaluated
- Information from the respiratory protection program, including observations made during fit testing and program evaluation, indicates a need for employee reevaluation
- A change occurs in workplace conditions (e.g., physical work effort, protective clothing, and temperature) that may result in a substantial increase in the physiological burden placed on an employee.

Respirator Fit Testing (see Appendix A for more information)
Before an employee is required to use any respirator with a negative or positive pressure tight-fitting face piece, the employee must be fit tested with the same make, model, style, and size of respirator that will be used. The Employer shall ensure that an employee using a tight-fitting face piece respirator is fit tested prior to initial use of the respirator, whenever a different respirator face piece (size, style, model or make) is used, and at least annually thereafter.
The employer has established a record of the qualitative and quantitative fit tests administered to employees including:
- The name or identification of the employee tested
- Type of fit test performed
- Specific make, model, style, and size of respirator tested
- Date of test
- The pass/fail results for QLFTs or the fit factor and strip chart recording or other recording of the test results for QNFTs

Additional fit tests will be conducted whenever the employee reports, or the employer, physician, supervisor, or program administrator makes visual observations of, changes in the employee's physical condition that could affect respirator fit.
Such conditions include, but are not limited to, facial scarring, dental changes, cosmetic surgery, or an obvious change in body weight.

If after passing a QLFT or QNFT, the employee notifies the employer’s program administrator, supervisor, or physician that the fit of the respirator is unacceptable, the employee shall be given a reasonable opportunity to select a different respirator face piece and to be retested.

Types of Fit Tests
The fit test shall be administered using an OSHA-accepted QLFT or QNFT protocol. The OSHA-accepted QLFT and QNFT protocols and procedures are contained in Appendix A of OSHA Standard 1910.134.
- QLFT may only be used to fit test negative pressure air-purifying respirators that must achieve a fit factor of 100 or less.
- If the fit factor, as determined through an OSHA-accepted QNFT protocol, is equal to or greater than 100 for tight-fitting half face pieces, or equal to or greater than 500 for tight-fitting full face pieces, the QNFT has been passed with that respirator.

- Fit testing of tight-fitting atmosphere-supplying respirators and tight-fitting powered air-purifying respirators shall be accomplished by performing quantitative or qualitative fit testing in the negative pressure mode, regardless of the mode of operation (negative or positive pressure) that is used for respiratory protection.
- Qualitative fit testing of these respirators shall be accomplished by temporarily converting the respirator user's actual face piece into a negative pressure respirator with appropriate filters, or by using an identical negative pressure air-purifying respirator face piece with the same sealing surfaces as a surrogate for the atmosphere-supplying or powered air-purifying respirator face piece.
- Quantitative fit testing of these respirators shall be accomplished by modifying the face piece to allow sampling inside the face piece in the breathing zone of the user, midway between the nose and mouth. This requirement shall be accomplished by installing a permanent sampling probe onto a surrogate face piece, or by using a sampling adapter designed to temporarily provide a means of sampling air from inside the face piece.
- Any modifications to the respirator face piece for fit testing shall be completely removed, and the face piece restored to NIOSH approved configuration, before that face piece can be used in the workplace.

Fit test records shall be retained for respirator users until the next fit test is administered. Written materials required to be retained shall be made available upon request to affected employees.

Respirator Operation and Use
Respirators will only be used following the respiratory protection safety procedures established in this program. The Operations and Use Manuals for each type of respirator will be maintained by the program administrator and be available to all qualified users.

Surveillance by the direct supervisor shall be maintained of work area conditions and degree of employee exposure or stress. When there is a change in work area conditions or degree of employee exposure or stress that may affect respirator effectiveness, the employer shall reevaluate the continued effectiveness of the respirator.

For continued protection of respirator users, the following general use rules apply:
- Users shall not remove respirators while in a hazardous environment
- Respirators are to be stored in sealed containers out of harmful atmospheres
- Store respirators away from heat and moisture
- Store respirators such that the sealing area does not become distorted or warped
- Store respirators such that the face piece is protected
- Face piece seal protection

The Employer does not permit respirators with tight-fitting face pieces to be worn by employees who have:
- Facial hair that comes between the sealing surface of the face piece and the face or that interferes with valve function; or
- Any condition that interferes with the face-to-face piece seal or valve function.

If an employee wears corrective glasses or goggles or other personal protective equipment, the employer shall ensure that such equipment is worn in a manner that does not interfere with the seal of the face piece to the face of the user.

Continuing Effectiveness of Respirators
The employer shall ensure that employees leave the respirator use area for the following:
- To wash their faces and respirator face pieces as necessary to prevent eye or skin irritation associated with respirator use
- If they detect vapor or gas breakthrough, changes in breathing resistance, or leakage of the face piece

- To replace the respirator or the filter, cartridge, or canister elements.

If the employee detects vapor or gas breakthrough, changes in breathing resistance, or leakage of the face piece, the employer will replace or repair the respirator before allowing the employee to return to the work area.

Procedures for IDLH atmospheres

For all IDLH atmospheres, the Employer shall ensure that:

- One employee or, when needed, more than one employee is located outside the IDLH atmosphere
- Visual, voice, or signal line communication is maintained between the employee(s) in the IDLH atmosphere and the employee(s) located outside the IDLH atmosphere
- The employee(s) located outside the IDLH atmosphere are trained and equipped to provide effective emergency rescue
- The employer or designee is notified before the employee(s) located outside the IDLH atmosphere enter the IDLH atmosphere to provide emergency rescue
- The employer or designee authorized to do so by the employer, once notified, provides necessary assistance appropriate to the situation

Employee(s) located outside the IDLH atmospheres will be equipped with:

- Pressure demand or other positive pressure SCBAs, or a pressure demand or other positive pressure supplied-air respirator with auxiliary SCBA; and either
- Appropriate retrieval equipment for removing the employee(s) who enter(s) these hazardous atmospheres where retrieval equipment would contribute to the rescue of the employee(s) and would not increase the overall risk resulting from entry; or
- Equivalent means for rescue where retrieval equipment is not required.

OSHA's General Industry Regulation, §1910.146 Permit-required confined spaces, contains requirements for practices and procedures to protect employees in general industry from the hazards of entry into permit-required confined spaces. This regulation does not apply to construction.

OSHA's Construction Safety and Health Regulations Part 1926 do not contain a permit-required confined space regulation. Subpart C, §1926.21 Safety training and education specifies training for personnel who are required to enter confined spaces and defines a "***confined or enclosed space***."

Gas and Vapor Contaminants

Gas and vapor contaminants can be classified according to their chemical characteristics. True gaseous contaminants are similar to air in that they possess the same ability to diffuse freely within an area or container. Nitrogen, chlorine, carbon monoxide, carbon dioxide and sulfur dioxide are examples.

Vapors are the gaseous state of substances that are liquids or solids at room temperature. They are formed when the solid or liquid evaporates. Gasoline, solvents and paint thinners are examples of liquids that evaporate easily, producing vapors.

In terms of chemical characteristics, gaseous contaminants may be classified as follows:

- **Inert Gases** —These include such true gases as helium, argon, neon, etc. Although they do not metabolize in the body, these gases represent a hazard because they can produce an oxygen deficiency by displacement of air.
- **Acidic Gases** —Often highly toxic, acidic gases exist as acids or produce acids by reaction with water. Sulfur dioxide, hydrogen sulfide and hydrogen chloride are examples.
- **Alkaline Gases** —These gases exist as alkalis or produce alkalis by reaction with water. Ammonia and phosphine are two examples.

In terms of chemical characteristics, vaporous contaminants may be classified as follows:

- **Organic Compounds** —Contaminants in this category can exist as true gases or vapors produced from organic liquids. Gasoline, solvents and paint thinners are examples.
- **Organometallic Compounds** —These are generally comprised of metals attached to organic groups. Tetraethyllead and organic phosphates are examples.

Hazard Assessment

Proper assessment of the hazard is the first important step to protection. This requires a thorough knowledge of processes, equipment, raw materials, end-products and by-products that can create an exposure hazard.

To determine an atmosphere's oxygen content or concentration levels of particulate and/or gaseous contaminants, air samples must be taken with proper sampling instruments during all conditions of operation. The sampling device and the type and frequency of sampling (spot testing or continuous monitoring) will be dictated by the exposure and operating conditions.

Breathing zone samples are recommended and sampling frequency should be sufficient to assess the average exposure under the variable operating and exposure conditions. Should contaminant concentrations exceed exposure limits recommended by the American Conference of Governmental Industrial Hygienists (**ACGIH**), OSHA or NIOSH, hazard control procedures must be implemented promptly. Exposure monitoring plays a critical role in the respirator selection process. The results from such tests will help you determine whether respiratory protection is needed and, if it is, the type of respirator required. Generally, respirator selection is based on three factors:

- The results of your atmospheric monitoring or sampling program;
- The accepted ACGIH, OSHA or NIOSH exposure limits for the substance(s) present;
- And the maximum use concentration (of a substance) for which a respirator can be used.

Exposure limits include ACGIH Threshold Limit Values (**TLVs**), OSHA Permissible Exposure Limits (**PELs**), NIOSH Recommended Exposure Levels (**RELs**) and AIHA Workplace Environmental Exposure Levels (**WEELs**). These values are guides for exposure concentrations that healthy individuals can normally tolerate for eight hours a day, five days a week without harmful effects. Unless otherwise noted, exposure limits are eight-hour, time-weighted-average (**TWA**) concentrations.

In general, gas and vapor exposure limits are expressed in ppm by volume (parts of contaminant per million parts of air), while particulate concentrations are expressed as mg/m3 (milligrams of

concentrations per cubic meter of air). For substances that can exist in more than one form (particulate or gaseous), concentrations are expressed in both values.
It is important to note that exposure limits and other exposure standards are constantly changing as more data is gathered about specific chemicals and substances. As such, you must be certain that you are using the most recent data when determining allowable exposure levels for employees.

Hazard Control
Hazard control should start at the process, equipment and plant design levels where contaminants can be effectively controlled at the outset. With operating processes, the problem becomes more difficult. In all cases, however, consideration should be given to the use of effective engineering controls to eliminate and/or reduce exposures to respiratory hazards. This includes consideration of process encapsulation or isolation, use of less toxic materials in the process and suitable exhaust ventilation, filters and scrubbers to control the effluents. Because it is sometimes not practical to maintain engineering controls that eliminate all airborne concentrations of contaminants, proper respiratory protective devices should be used whenever such protection is required.

Hazard Assessment or Hazard Certification sheet example is on the following page.
Even if you have a written RP Program and complete training records, OSHA will ask for a hazard certification or assessment form on where or why you need RP.For example, if you were required to don SCBA to change a chlorine cylinder once a week, OSHA would request to see how that task was evaluated and certified.

RP Cleaning and Disinfecting *(See Appendix B for more information)*

The employer shall provide each respirator user with a respirator that is clean, sanitary, and in good working order. The employer shall ensure that respirators are cleaned and disinfected using the Standard Operating Procedure SOP: Cleaning and Disinfecting.

The respirators shall be cleaned and disinfected when:

- Respirators issued for the exclusive use of an employee shall be cleaned and disinfected as often as necessary to be maintained in a sanitary condition.
- Respirators issued to more than one employee shall be cleaned and disinfected before being worn by different individuals.
- Respirators maintained for emergency use shall be cleaned and disinfected after each use.
- Respirators used in fit testing and training shall be cleaned and disinfected after each use.

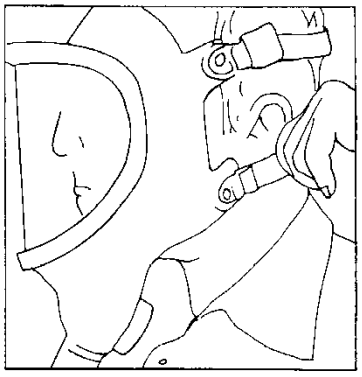
Cleaning and Storage of respirators assigned to specific employees is the responsibility of that employee.

Respirator Inspection

All respirators/SCBAs, both available for "**General Use**" and those on "**Permanent Check-out**", will be inspected after each use and at least monthly. Should any defects be noted, the respirator/SCBA will be taken to the program Administrator. Damaged Respirators will be either repaired or replaced. The inspection of respirators loaned on "**Permanent Check-out**" is the responsibility of that trained employee.

Respirators shall be inspected as follows:

- All respirators used in routine situations shall be inspected before each use and during cleaning.
- All respirators maintained for use in emergency situations shall be inspected at least monthly and in accordance with the manufacturer's recommendations, and shall be checked for proper function before and after each use.
- Emergency escape-only respirators shall be inspected before being carried into the workplace for use.

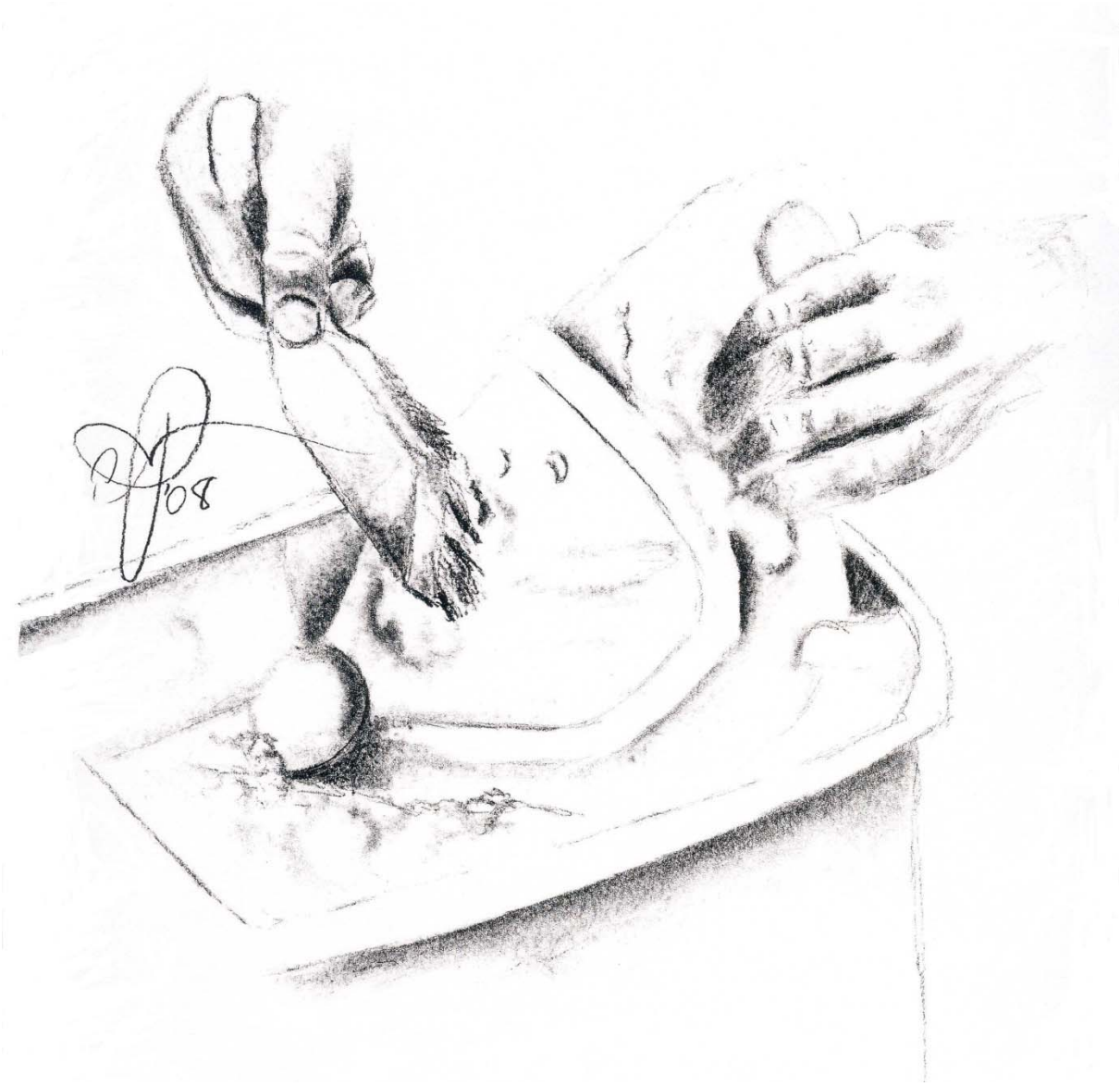


Respirator inspections include the following:

- A check of respirator function, tightness of connections, and the condition of the various parts including, but not limited to, the face piece, head straps, valves, connecting tube, and cartridges, canisters or filters
- Check of elastomeric parts for pliability and signs of deterioration.
- Self-contained breathing apparatus shall be inspected monthly. Air and oxygen cylinders shall be maintained in a fully charged state and shall be recharged when the pressure falls to 90% of the manufacturer's recommended pressure level. The employer shall determine that the regulator and warning devices function properly

For Emergency Use Respirators the additional requirements apply:

- Certify the respirator by documenting the date the inspection was performed, the name (or signature) of the person who made the inspection, the findings, required remedial action, and a serial number or other means of identifying the inspected respirator.
- Provide this information on a tag or label that is attached to the storage compartment for the respirator, is kept with the respirator, or is included in inspection reports stored as paper or electronic files. This information shall be maintained until replaced following a subsequent certification.



Respirator Storage

Respirators are to be stored as follows:

- All respirators shall be stored to protect them from damage, contamination, dust, sunlight, extreme temperatures, excessive moisture, and damaging chemicals, and they shall be packed or stored to prevent deformation of the face piece and exhalation valve.
- **Emergency Respirators shall be**:
  - Kept accessible to the work area;
  - Stored in compartments or in covers that are clearly marked as containing emergency respirators; and
  - Stored in accordance with any applicable manufacturer instructions.

Repair of Respirators

Respirators that fail an inspection or are otherwise found to be defective will be removed from service to be discarded, repaired or adjusted in accordance with the following procedures:

- Repairs or adjustments to respirators are to be made only by persons appropriately trained to perform such operations and shall use only the respirator manufacturer's NIOSH-approved parts designed for the respirator;
- Repairs shall be made according to the manufacturer's recommendations and specifications for the type and extent of repairs to be performed; and
- Reducing and admission valves, regulators, and alarms shall be adjusted or repaired only by the manufacturer or a technician trained by the manufacturer.

Breathing Air Quality and Use

The employer shall ensure that compressed air, compressed oxygen, liquid air, and liquid oxygen used for respiration accords with the following specifications:

- Compressed and liquid oxygen shall meet the United States Pharmacopoeia Requirements for medical or breathing oxygen; and
- Compressed breathing air shall meet at least the requirements for Grade D breathing air described in ANSI/Compressed Gas Association Commodity Specification for Air, G-7.1-1989, to include:
- Oxygen content (**v/v**) of 19.5-23.5%;
- Hydrocarbon (condensed) content of 5 milligrams per cubic meter of air or less;
- Carbon monoxide content of 10 ppm or less;
- Carbon dioxide content of 1,000 ppm or less; and
- Lack of noticeable odor.
- Compressed oxygen will not be used in atmosphere-supplying respirators that have previously used compressed air.
- Oxygen concentrations greater than 23.5% are used only in equipment designed for oxygen service or distribution.

Cylinders used to supply breathing air to respirators meet the following requirements:

- Cylinders are tested and maintained as prescribed in the Shipping Container Specification Regulations of the Department of Transportation (49 CFR part 173 and part 178).
- Cylinders of purchased breathing air have a certificate of analysis from the supplier that the breathing air meets the requirements for Grade D breathing air.
- Moisture content in breathing air cylinders does not exceed a dew point of -50 deg. F (-45.6 deg. C) at 1 atmosphere pressure.
- Breathing air couplings are incompatible with outlets for nonrespirable worksite air or other gas systems. No asphyxiating substance shall be introduced into breathing air lines.
- Breathing gas containers shall be marked in accordance with the NIOSH respirator certification standard, 42 CFR part 84.

Summary

Following this training session, employees should:

- Wear the respirator assigned to him or her
- Always check for fit before wearing
- Always check for damage and deterioration before wearing
- Know when to replace canisters and cartridges
- Practice maneuvering with a respirator
- Store carefully in the proper location.

Glossary

ABIOGENESIS: The concept of spontaneous generation (that life can come from non-life). This idea was refuted by Pasteur.

ABIOTIC: The non-living components of an organism's environment. The term abiotic is also used to denote a process which is not facilitated by living organisms.

ABORAL: Pertaining to the region of the body opposite that of the mouth. Normally used to describe radially symmetrical animals.

ABSCISIC ACID (ABA): A plant hormone that generally acts to inhibit growth, promote dormancy, and help the plant withstand stressful conditions.

ABSENCE OF OXYGEN: The complete absence of oxygen in water described as Anaerobic.

ABSORPTION SPECTRUM: The range of a material's ability to absorb various wavelengths of light. The absorption spectrum is studied to evaluate the function of photosynthetic pigments.

ACCURACY: How closely an instrument measures the true or actual value.

ACCESSORY PIGMENT: A photosynthetic pigment which absorbs light and transfers energy to chlorophylls during photosynthesis. Because accessory pigments have different absorption optima than chlorophylls, presence of accessory pigments allows photosynthetic systems to absorb light more efficiently than would be possible otherwise.

ACELLULAR: Not within cells. Sometimes used as a synonym for unicellular (but multinucleate). Unicellular also pertains to single: celled organisms.

ACETYL COA: Acetyl CoenzymeA is the entry compound for the Krebs cycle in cellular respiration; formed from a fragment of pyruvic acid attached to a coenzyme.

ACETYLCHOLINE: A neurotransmitter substance that carries information across vertebrate neuromuscular junctions and some other synapses.

ACID: Slowly add the acid to water while stirring. An operator should not mix acid and water or acid to a strong base.

ACID AND BASE ARE MIXED: When an acid and a base are mixed, an explosive reaction occurs and decomposition products are created under certain conditions.

ACID RAIN: Rain that is excessively acidic due to the presence of acid: causing pollutants in the atmosphere. Pollutants include nitrogen and sulfur oxides due to burning of coal and oil.

ACIDOSIS: A condition whereby the hydrogen ion concentration of the tissues is increased (and pH decreased). Respiratory acidosis is due to the retention of CO_2; metabolic acidosis by retention of acids due either to kidney failure or diarrhea.

ACOELOMATE: Lacking a coelom.

ACQUIRED IMMUNITY: Results from exposure to foreign substances or microbes (also called natural immunity).

ACROSOME: An organelle at the tip of a sperm cell that helps the sperm penetrate the egg.

ACTH (adrenocorticotrophic hormone): A proteineinaceous hormone from the anterior pituitary that stimulates the adrenal cortex. Used to stimulate the production of cortisol.

ACTIN: A globular protein that links into chains, two of which twist helically about each other, forming microfilaments in muscle and other contractile elements in cells.

ACTION POTENTIAL: The stimulus- triggered change in the membrane potential of an excitable cell, caused by selective opening and closing of ion channels.

ACTION SPECTRUM: A graph which illustrates the relationship between some biological activity and wavelength of light.

ACTIVATING ENZYME: An enzyme that couples a low-energy compound with ATP to yield a high-energy derivative.

ACTIVATION ENERGY: In a chemical reaction, the initial investment required to energize the bonds of the reactants to an unstable transition state that precedes the formation of the products.

ACTIVE SITE: That specific portion of an enzyme that attaches to the substrate by means of weak chemical bonds.

ACTIVE TRANSPORT: The movement of a substance across a biological membrane against its concentration or electrochemical gradient with the help of energy input and specific transport proteins.

ACTIVATED SLUDGE: The biologically active solids in an activated sludge process wastewater treatment plant.

ACTIVATED SLUDGE PROCESS: A biological wastewater treatment process in which a mixture of wastewater and biologically enriched sludge is mixed and aerated to facilitate aerobic decomposition by microbes.

ADAPTATION: Any genetically controlled characteristic that increases an organism's fitness, usually by helping the organism to survive and reproduce in the environment it inhabits.

ADAPTIVE RADIATION: This refers to the rapid evolution of one or a few forms into many different species that occupy different habitats within a new geographical area.

ADHESION: In chemistry, the phenomenon whereby one substance tends to cling to another substance. Water molecules exhibit adhesion, especially toward charged surfaces.

ADP (Adenosine diphosphate): A doubly phosphorylated organic compound that can be further phosphorylated to form ATP.

ADRENAL GLAND: An endocrine gland located adjacent to the kidney in mammals. It is composed of an outer cortex, and a central medulla, each involved in different hormone: mediated phenomena.

ADRENALIN: A hormone produced by the pituitary that stimulates the adrenal cortex.

ADSORB: Hold on a surface.

ADSORPTION: *Not to be confused with absorption.* Adsorption is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a film of molecules or atoms (the adsorbate). It is different from absorption, in which a substance diffuses into a liquid or solid to form a solution. The term sorption encompasses both processes, while desorption is the reverse process. Adsorption is present in many natural physical, biological, and chemical systems, and is widely used in industrial applications such as activated charcoal, synthetic resins, and water purification. Adsorption, ion exchange, and chromatography are sorption processes in which certain adsorbates are selectively transferred from the fluid phase to the surface of insoluble, rigid particles suspended in a vessel or packed in a column. Similar to surface tension, adsorption is a consequence of surface energy. In a bulk material, all the bonding requirements (be they ionic, covalent, or metallic) of the constituent atoms of the material are filled by other atoms in the material. However, atoms on the surface of the adsorbent are not wholly surrounded by other adsorbent atoms, and therefore can attract adsorbates. The exact nature of the bonding depends on the details of the species involved, but the adsorption process is generally classified as physisorption (characteristic of weak van der Waals forces) or chemisorption (characteristic of covalent bonding).

ADVANCED: New, unlike the ancestral condition.

AERATION: The addition of air or oxygen to water or wastewater, usually by mechanical means, to increase dissolved oxygen levels and maintains aerobic conditions.

AEROBIC: The condition of requiring oxygen; an aerobe is an organism which can live and grow only in the presence of oxygen.

AEROBIC DIGESTION: Sludge stabilization process involving direct oxidation of biodegradable matter and oxidation of microbial cellular material.

AGE STRUCTURE: The relative numbers of individuals of each age in a population.

AGONISTIC BEHAVIOR: A type of behavior involving a contest of some kind that determines which competitor gains access to some resource, such as food or mates.

AIDS (acquired immune deficiency syndrome): A condition in which the body's helper T lymphocytes are destroyed, leaving the victim subject to opportunistic diseases.

AIR GAP SEPARATION: A physical separation space that is present between the discharge vessel and the receiving vessel; for an example, a kitchen faucet.

AIR ENTRAINMENT: The dissolution or inclusion of air bubbles into water.

ALCOHOL: Any of a class of organic compounds in which one or more - OH groups are attached to a carbon compound.

ALDEHYDE: An organic molecule with a carbonyl group located at the end of the carbon skeleton.

ALGAE: Microscopic plants that are free-living and usually live in water. They occur as single cells floating in water, or as multicellular plants like seaweed or strands of algae that attach to rocks.

ALPHA AND BETA RADIOACTIVITY: Represent two common forms of radioactive decay. Radioactive elements have atomic nuclei so heavy that the nucleus will break apart, or disintegrate spontaneously. When decay occurs, high-energy particles are released. These high-energy particles are called radioactivity. Although radioactivity from refined radioactive elements can be dangerous, it is rare to find dangerous levels of radioactivity in natural waters. An alpha particle is a doubly-charged helium nucleus comprised of two protons, two neutrons, and no electrons. A beta particle is a high-speed electron. Alpha particles do not penetrate matter easily, and are stopped by a piece of paper. Beta particles are much more penetrating and can pass through a millimeter of lead.

ALKALINE: Having a pH of more than 7. Alkaline solutions are also said to be basic.

ALKALINITY: Alkalinity or AT is a measure of the ability of a solution to neutralize acids to the equivalence point of carbonate or bicarbonate. Alkalinity is closely related to the acid neutralizing capacity (ANC) of a solution and ANC is often incorrectly used to refer to alkalinity. However, the acid neutralizing capacity refers to the combination of the solution and solids present (e.g., suspended matter, or aquifer solids), and the contribution of solids can dominate the ANC (see carbonate minerals below). The alkalinity is equal to the stoichiometric sum of the bases in solution. In the natural environment carbonate alkalinity tends to make up most of the total alkalinity due to the common occurrence and dissolution of carbonate rocks and presence of carbon dioxide in the atmosphere. Other common natural components that can contribute to alkalinity include borate, hydroxide, phosphate, silicate, nitrate, dissolved ammonia, the conjugate bases of some organic acids and sulfide. Solutions produced in a laboratory may contain a virtually limitless number of bases that contribute to alkalinity. Alkalinity is usually given in the unit mEq/L (milliequivalent per liter). Commercially, as in the pool industry, alkalinity might also be given in the unit ppm or parts per million. Alkalinity is sometimes incorrectly used interchangeably with basicity. For example, the pH of a solution can be lowered by the addition of CO_2. This will reduce the basicity; however, the alkalinity will remain unchanged.

ALLANTOIS: One of the four extraembryonic membranes found associated with developing vertebrates; it serves in gas exchange and as a repository for the embryo's nitrogenous waste. In humans, the allantois is involved in early blood formation and development of the urinary bladder.

ALLELE: Alternate forms of a gene which may be found at a given location (locus) on members of a homologous set of chromosomes. Structural variations between alleles may lead to different phenotypes for a given trait.

ALLOMETRIC: The variation in the relative rates of growth of various parts of the body, which helps shape the organism.

ALLOPATRIC SPECIATION: A type of speciation which occurs when a population becomes segregated into two populations by some sort of geographic barrier (also called geographic speciation). This phenomenon is presumed to have been the mechanism whereby many species of organisms evolved.

ALLOPOLYPLOID: A common type of polyploid species resulting from two different species interbreeding and combining their chromosomes.

ALL-OR-NONE: (event) An action that occurs either completely or not at all, such as the generation of an action potential by a neuron.

ALLOSTERIC ENZYME: An enzyme that can exist in two or more conformations.

ALLOSTERIC SITE: A receptor on an enzyme molecule which is remote from the active site. Binding of the appropriate molecule to the allosteric site changes the conformation of the active site, making it either more or less receptive to the substrate.

ALPHA HELIX: A spiral shape constituting one form of the secondary structure of proteins, arising from a specific hydrogen: bonding structure.

ALTERNATION OF GENERATIONS: Occurrences of a multicellular diploid form, the sporophyte, with a multicellular haploid form, the gametophyte.

ALTERNATIVE DISINFECTANTS: Disinfectants - other than chlorination (halogens) - used to treat water, e.g. ozone, ultraviolet radiation, chlorine dioxide, and chloramine. There is limited experience and scientific knowledge about the by-products and risks associated with the use of alternatives.

ALTRUISM: The willingness of an individual to sacrifice its fitness for the benefit of another.

ALUMINUM SULFATE: The chemical name for Alum. The molecular formula of Alum is $Al_2(SO_4)3{\sim}14H_2O$. It is a cationic polymer.

ALVEOLUS: One of the dead-end, multilobed air sacs that constitute the gas exchange surface of the lungs.

AMINO ACID: An organic molecule possessing a carboxyl (COOH) and amino group. Amino acids serve as the monomers of polypeptides and proteins.

AMINO GROUP: A functional group consisting of a nitrogen atom bonded to two hydrogens; can act as a base in solution, accepting a hydrogen ion and acquiring a charge of +1.

AMINOACYL: tRNA synthetases- A family of enzymes, at least one for each amino acid, that catalyze the attachment of an amino acid to its specific tRNA molecule.

AMOEBA: Amoeba (sometimes amœba or ameba, plural amoebae) is a genus of protozoa that moves by means of pseudopods, and is well-known as a representative unicellular organism. The word amoeba or ameba is variously used to refer to it and its close relatives, now grouped as the Amoebozoa, or to all protozoa that move using pseudopods, otherwise termed amoeboids.

AMOEBOID: (cell) A cell which has the tendency to change shape by protoplasmic flow. (movement) A streaming locomotion characteristic of Amoeba and other protists, as well as some individual cells, such as white blood cells, in animals.

AMMONIA: A chemical made with Nitrogen and Hydrogen and used with chlorine to disinfect water. Most ammonia in water is present as the ammonium ion rather than as ammonia.

AMP (Adenosine monophosphate): A singly phosphorylated organic compound that can be further phosphorylated to form ADP.

AMYLASE: A starch-digesting enzyme.

ANABOLISM: A metabolic pathway of biosynthesis that consumes energy to build a large molecule from simpler ones.

ANAEROBIC: Without oxygen. An organism which lives in the absence of oxygen is called an anaerobe. An abnormal condition in which color and odor problems are most likely to occur.

ANAEROBIC CONDITIONS: When anaerobic conditions exist in either the metalimnion or hypolimnion of a stratified lake or reservoir, water quality problems may make the water unappealing for domestic use without costly water treatment procedures. Most of these problems are associated with Reduction in the stratified waters.

ANAEROBIC DIGESTION: Sludge stabilization process where the organic material in biological sludges are converted to methane and carbon dioxide in an airtight reactor.

ANAGENESIS: A pattern of evolutionary change involving the transformation of an entire population, sometimes to a state different enough from the ancestral population to justify renaming it as a separate species; also called phyletic.

ANALOGOUS: Characteristics of organisms which are similar in function (and often in structure) but different in embryological and/or evolutionary origins.

ANALYST: The analyst must have at least 2 years of college lecture and laboratory course work in microbiology or a closely related field. The analyst also must have at least 6 months of continuous bench experience with environmental protozoa detection techniques and IFA microscopy, and must have successfully analyzed at least 50 water and/or wastewater samples for *Cryptosporidium* and *Giardia*. Six months of additional experience in the above areas may be substituted for two years of college.

ANCESTRAL TRAIT: Trait shared by a group of organisms as a result of descent from a common ancestor.

ANEUPLOIDY: A chromosomal aberration in which certain chromosomes are present in extra copies or are deficient in number.

ANION: A negatively charged ion.

ANISOGAMOUS: Reproducing by the fusion of gametes that differ only in size, as opposed to gametes that are produced by oogamous species. Gametes of oogamous species, such as egg cells and sperm, are highly differentiated.

ANNUAL: A plant that completes its entire life cycle in a single year or growing season.

ANOXIC: A biological environment that is deficient in molecular oxygen, but may contain chemically bound oxygen, such as nitrates and nitrites.

ANTERIOR: Referring to the head end of a bilaterally symmetrical animal.

ANTHROPOMORPHISM: Attributing a human characteristic to an inanimate object or a species other than a human.

ANTIBIOTIC: A chemical that kills or inhibits the growth of bacteria, often via transcriptional or translational regulation.

ANTIBODY: A protein, produced by the B lymphocytes of the immune system that binds to a particular antigen.

ANTICODON: The specialized base triplet on one end of a tRNA molecule that associates with a particular complementary codon on an mRNA molecule during protein synthesis.

ANTIDIURETIC HORMONE: A hormone important in osmoregulation (it acts to reduce the elimination of water from the body.

ANTIGEN: A foreign macromolecule that does not belong to the host organism and that elicits an immune response.

APOMORPHIC CHARACTER: A derived phenotypic character, or homology, that evolved after a branch diverged from a phylogenetic tree.

APOSEMATIC COLORATION: Serving as a warning, with reference particularly to colors and structures that signal possession of defensive device.

AQUEOUS SOLUTION: A solution in which water is the solvent.

ARCHAEBACTERIA: A lineage of prokaryotes, represented today by a few groups of bacteria inhabiting extreme environments. Some taxonomists place archaebacteria in their own kingdom, separate from the other bacteria.

ARCHENTERON: The endoderm-lined cavity formed during the gastrulation process that develops into the digestive tract of the animal.

ARISTOTLE: A Greek philosopher often credited as the first to use empirical and deductive methods in logic.

ARTIFICIAL SELECTION: The selective breeding of domesticated plants and animals to encourage the occurrence of desirable traits.

AS: The chemical symbol of Arsenic.

AS NITROGEN: An expression that tells how the concentration of a chemical is expressed mathematically. The chemical formula for the nitrate ion is NO_3 , with a mass of 62. The concentration of nitrate can be expressed either in terms of the nitrate ion or in terms of the principal element, nitrogen. The mass of the nitrogen atom is 14. The ratio of the nitrate ion mass to the nitrogen atom mass is 4.43. Thus a concentration of 10 mg/L nitrate expressed as nitrogen would be equivalent to a concentration of 44.3 mg/L nitrate expressed as nitrate ion. When dealing with nitrate numbers it is very important to know how numeric values are expressed.

ASCUS: The elongate spore sac of a fungus of the Ascomycota group.

ASEXUAL: A type of reproduction involving only one parent that produces genetically identical offspring by budding or division of a single cell or the entire organism into two or more parts.

ASSORTATIVE MATING: A type of nonrandom mating in which mating partners resemble each other in certain phenotypic characters.

ASYMMETRIC CARBON: A carbon atom covalently bonded to four different atoms or groups of atoms.

ATOM: The general definition of an ion is an atom with a positive or negative charge. Electron is the name of a negatively charged atomic particle.

ATOMIC NUMBER: The number of protons in the nucleus of an atom, unique for each element.

ATOMIC THEORY: The physical theory of the structure, properties and behavior of the atom.

ATOMIC WEIGHT: The total atomic mass, which is the mass in grams of one mole of the atom (relative to that of 12C, which is designated as 12).

ATP (Adenosine triphosphate): A triply phosphorylated organic compound that functions as "energy currency" for organisms, thus allowing life forms to do work; it can be hydrolyzed in two steps (first to ADP and then to AMP) to liberate 7.3 Kcal of energy per mole during each hydrolysis.

ATPASE: An enzyme that functions in producing or using ATP.

AUTOGENOUS MODEL: A hypothesis which suggests that the first eukaryotic cells evolved by the specialization of internal membranes originally derived from prokaryotic plasma membranes.

AUTOIMMUNE DISEASE: An immunological disorder in which the immune system goes awry and turns against itself.

AUTONOMIC NERVOUS SYSTEM: A subdivision of the motor nervous system of vertebrates that regulates the internal environment; consists of the sympathetic and parasympathetic subdivisions.

AUTOPOLYPLOID: A type of polyploid species resulting from one species doubling its chromosome number to become tetraploids, which may self-fertilize or mate with other tetraploids.

AUTOSOME: Chromosomes that are not directly involved in determining sex.

AUTOTROPH: An organism which is able to make organic molecules from inorganic ones either by using energy from the sun or by oxidizing inorganic substances.

AUXIN: One of several hormone compounds in plants that have a variety of effects, such as phototropic response through stimulation of cell elongation, stimulation of secondary growth, and development of leaf traces and fruit.

AUXOTROPH: A nutritional mutant that is unable to synthesize and that cannot grow on media lacking certain essential molecules normally synthesized by wild-type strains of the same species.

AXON: A typically long outgrowth, or process, from a neuron that carries nerve impulses away from the cell body toward target cells.

AXONEME: An internal flagellar structure that occurs in some protozoa, such as *Giardia*, *Spironucleous*, and *Trichonmonas*.

B-CELL LYMPHOCYTE: A type of lymphocyte that develops in the bone marrow and later produces antibodies, which mediate humoral immunity.

BACKFLOW: To reverse the natural and normal directional flow of a liquid, gases, or solid substances back in to the public potable (drinking) water supply. This is normally an undesirable effect.

BACKFLOW PREVENTION: To stop or prevent the occurrence of, the unnatural act of reversing the normal direction of the flow of liquid, gases, or solid substances back in to the public potable (drinking) water supply. See Cross-connection control.

BACKSIPHONAGE: A liquid substance that is carried over a higher point. It is the method by which the liquid substance may be forced by excess pressure over or into a higher point.

BACTERIA: Small, one-celled animals too small to be seen by the naked eye. Bacteria are found everywhere, including on and in the human body. Humans would be unable to live without the bacteria that inhabit the intestines and assist in digesting food. Only a small percentage of bacteria cause disease in normal, healthy humans. Other bacteria can cause infections if they get into a cut or wound. Bacteria are the principal concern in evaluating the microbiological quality of drinking water, because some of the bacteria-caused diseases that can be transmitted by drinking water are potentially life-threatening.

BACTERIOPHAGE: A bacteriophage (from 'bacteria' and Greek phagein, 'to eat') is any one of a number of viruses that infect bacteria. The term is commonly used in its shortened form, phage. Typically, bacteriophages consist of an outer protein hull enclosing genetic material. The genetic material can be ssRNA (single stranded RNA), dsRNA, ssDNA, or dsDNA between 5 and 500 kilo base pairs long with either circular or linear arrangement. Bacteriophages are much smaller than the bacteria they destroy - usually between 20 and 200 nm in size.

BACTERIUM: A unicellular microorganism of the Kingdom Monera. Bacteria are prokaryotes; their cells have no true nucleus. Bacteria are classified into two groups based on a difference in cell walls, as determined by Gram staining.

BALANCED POLYMORPHISM: A type of polymorphism in which the frequencies of the coexisting forms do not change noticeably over many generations.

BARR BODY: The dense object that lies along the inside of the nuclear envelope in cells of female mammals, representing the one inactivated X chromosome.

BASAL BODY: A cell structure identical to a centriole that organizes and anchors the microtubule assembly of a cilium or flagellum.

BASE PAIRING: Complementary base pairing refers to the chemical affinities between specific base pairs in a nucleic acid: adenine always pairs with thymine, and guanine always pairs with cytosine. In pairing between DNA and RNA, the uracil of RNA always pairs with adenine. Complementary base pairing is not only responsible for the DNA double helix, but it is also essential for various in vitro techniques such as PCR (polymerase chain reaction). Complementary base pairing is also known as Watson-Crick pairing.

BASE: A substance that reduces the hydrogen ion concentration in a solution.

BASEMENT MEMBRANE: The floor of an epithelial membrane on which the basal cells rest.

BASIDIUM: The spore-bearing structure of Basidiomycota.

BATESIAN MIMICRY: A type of mimicry in which a harmless species looks like a different species that is poisonous or otherwise harmful to predators.

BEHAVIORAL ECOLOGY: A heuristic approach based on the expectation that Darwinian fitness (reproductive success) is improved by optimal behavior.

BELT PRESS: A dewatering device utilizing two opposing synthetic fabric belts, revolving over a series of rollers to "squeeze" water from the sludge.

BENIGN TUMOR: A noncancerous abnormal growth composed of cells that multiply excessively but remain at their place of origin in the body.

BENTHIC: Pertaining to the bottom region of an aquatic environment.

BENCH TEST: A small-scale test or study used to determine whether a technology is suitable for a particular application.

BEST AVAILABLE TECHNOLOGY ECONOMICALLY ACHIEVABLE (BAT): A level of technology based on the best existing control and treatment measures that are economically achievable within the given industrial category or subcategory.

BEST MANAGEMENT PRACTICES (BMPs): Schedules of activities, prohibitions of practices, maintenance procedures, and other management practices to prevent or reduce the pollution of waters of the U.S. BMPs also include treatment requirements, operating procedures and practices to control plant site runoff, spillage or leaks, sludge or waste disposal, or drainage from raw material storage.

BEST PRACTICABLE CONTROL TECHNOLOGY CURRENTLY AVAILABLE (BPT):
A level of technology represented by the average of the best existing wastewater treatment performance levels within an industrial category or subcategory.

BEST PROFESSIONAL JUDGMENT (BPJ): The method used by a permit writer to develop technology-based limitations on a case-by-case basis using all reasonably available and relevant data.

BETA PLEATED SHEET: A zigzag shape, constituting one form of the secondary structure of proteins formed of hydrogen bonds between polypeptide segments running in opposite directions.

BILATERAL SYMMETRY: The property of having two similar sides, with definite upper and lower surfaces and anterior and posterior ends. The Bilateria are members of the branch of Eumetazoa (Kingdom Animalia) which possess bilateral symmetry.

BILE: A mixture of substances containing bile salts, which emulsify fats and aid in their digestion and absorption.

BINARY FISSION: The kind of cell division found in prokaryotes, in which dividing daughter cells each receive a copy of the single parental chromosome.

BINOMIAL NOMENCLATURE: Consisting of two names. In biology, each organism is given a *genus* name and a species name (i.e., the human is Homo sapiens.

BIOCHEMICAL OXYGEN DEMAND (BOD): The BOD test is used to measure the strength of wastewater. The BOD of wastewater determines the milligrams per liter of oxygen required during stabilization of decomposable organic matter by aerobic bacteria action. Also, the total milligrams of oxygen required over a five-day test period to biologically assimilate the organic contaminants in one liter of wastewater maintained at 20 degrees Centigrade.

BIOGENESIS: A central concept of biology, that living organisms are derived from other living organisms (contrasts to the concept of abiogenesis, or spontaneous generation, which held that life could be derived from inanimate material).

BIOGEOCHEMICAL CYCLE: A circuit whereby a nutrient moves between both biotic and abiotic components of ecosystems.

BIOGEOGRAPHY: The study of the past and present distribution of species.

BIOLOGICAL MAGNIFICATION: Increasing concentration of relatively stable chemicals as they are passed up a food chain from initial consumers to top predators.

BIOLOGICAL SPECIES: A population or group of populations whose members have the potential to interbreed. This concept was introduced by Ernst Mayr.

BIOMASS: The total weight of all the organisms, or of a designated group of organisms, in a given area

BIOME: A large climatic region with characteristic sorts of plants and animals.

BIOSOLIDS: Solid organic matter recovered from municipal wastewater treatment that can be beneficially used, especially as a fertilizer. “Biosolids” are solids that have been stabilized within the treatment process, whereas “sludge” has not.

BIOSPHERE: The region on and surrounding the earth which is capable of supporting life. Theoretically, the concept may be ultimately expanded to include other regions of the universe.

BMR: The basal metabolic rate is the minimal energy (in kcal) required by a homeotherm to fuel itself for a given time. Measured within the thermoneutral zone for a postabsorptive animal at rest.

BODY FEED: Coating or bulking material added to the influent of material to be treated. This adds “body” to the material during filtration cycle.

BREAK POINT CHLORINATION: The process of chlorinating the water with significant quantities of chlorine to oxidize all contaminants and organic wastes and leave all remaining chlorine as free chlorine.

BROMINE: Chemical disinfectant (HALOGEN) that kills bacteria and algae. This chemical disinfectant has been used only on a very limited scale for water treatment because of its handling difficulties. This chemical causes skin burns on contact, and a residual is difficult to obtain.

BUFFER: Chemical that resists pH change, e.g. sodium bicarbonate

BULKING SLUDGE: A poor or slow settling activated sludge that results from the prevalence of filamentous organisms. A phenomenon that occurs in activated sludge plants whereby the sludge occupies excessive volumes and will not concentrate readily. This condition refers to a decrease in the ability of the sludge to settle and consequent loss over the settling tank weir. Bulking in activated sludge aeration tanks is caused mainly by excess suspended solids (SS) content. Sludge bulking in the final settling tank of an activated sludge plant may be caused by improper balance of the BOD load, SS concentration in the mixed liquor, or the amount of air used in aeration.

Ca: The chemical symbol for calcium.

CADMIUM: A contaminant that is usually not found naturally in water or in very small amounts.

CAKE: Dewatered sludge material with a satisfactory solids concentration to allow handling as a solid material.

CALCIUM HARDNESS: A measure of the calcium salts dissolved in water.

CALCIUM ION: Is divalent because it has a valence of +2.

CALCIUM, MAGNESIUM AND IRON: The three elements that cause hardness in water.

$CaOCl_2.4H_2O$: The molecular formula of Calcium hypochlorite.

CARBON DIOXIDE GAS: The pH will decrease and alkalinity will change as measured by the Langelier index after pumping carbon dioxide gas into water.

CARBONATE HARDNESS: Carbonate hardness is the measure of Calcium and Magnesium and other hard ions associated with carbonate ($CO_3$2-) and bicarbonate (HCO_3-) ions contained in a solution, usually water. It is usually expressed either as parts per million (ppm or mg/L), or in degrees (KH - from the German "Karbonathärte"). One German degree of carbonate hardness is equivalent to about 17.8575 mg/L.

Both measurements (mg/L or KH) are usually expressed "as $CaCO_3$" – meaning the amount of hardness expressed as if calcium carbonate was the sole source of hardness. Every bicarbonate ion only counts for half as much carbonate hardness as a carbonate ion does. If a solution contained 1 liter of water and 50 mg $NaHCO_3$ (baking soda), it would have a carbonate hardness of about 18 mg/L as $CaCO_3$. If you had a liter of water containing 50 mg of Na_2CO_3, it would have a carbonate hardness of about 29 mg/L as $CaCO_3$. Carbonate hardness supplements non-carbonate (a.k.a. "permanent") hardness where hard ions are associated with anions such as Chloride that do not precipitate out of solution when heated. Carbonate hardness is removed from water through the process of softening. Softening can be achieved by adding lime in the form of $Ca(OH)_2$, which reacts first with CO_2 to form calcium carbonate precipitate, reacts next with multi-valent cations to remove carbonate hardness, then reacts with anions to replace the non-carbonate hardness due to multi-valent cations with non-carbonate hardness due to calcium. The process requires recarbonation through the addition of carbon-dioxide to lower the pH which is raised during the initial softening process.

CARBONATE, BICARBONATE AND HYDROXIDE: Chemicals that are responsible for the alkalinity of water.

CAROLUS LINNAEUS: Swedish botanist and originator of the binomial nomenclature system of taxonomic classification

CATHODIC PROTECTION: An operator should protect against corrosion of the anode and/or the cathode by painting the copper cathode. Cathodic protection interrupts corrosion by supplying an electrical current to overcome the corrosion-producing mechanism. Guards against stray current corrosion.

CAUSTIC: NaOH (also called Sodium Hydroxide) is a strong chemical used in the treatment process to neutralize acidity, increase alkalinity or raise the pH value.

CAUSTIC SODA: Also known as sodium hydroxide and is used to raise pH.

CENTRATE: The liquid remaining after solids have been removed in a centrifuge.

CENTRIFUGE: A dewatering device relying on centrifugal force to separate particles of varying density such as water and solids.

CENTRIFUGAL FORCE: That force when a ball is whirled on a string that pulls the ball outward. On a centrifugal pump, it is that force which throws water from a spinning impeller.

CENTRIFUGAL PUMP: A pump consisting of an impeller fixed on a rotating shaft and enclosed in a casing, having an inlet and a discharge connection. The rotating impeller creates pressure in the liquid by the velocity derived from centrifugal force.

CHAIN OF CUSTODY (COC): A record of each person involved in the possession of a sample from the person who collects the sample to the person who analyzes the sample in the laboratory.

CHECK VALVE: Allows water to flow in only one direction.

CHELATION: A chemical process used to control scale formation in which a chelating agent "captures" scale-causing ions and holds them in solution.

CHEMICAL FEED RATE: Chemicals are added to the water in order to improve the subsequent treatment processes. These may include pH adjusters and coagulants. Coagulants are chemicals, such as alum, that neutralize positive or negative charges on small particles, allowing them to stick together and form larger particles that are more easily removed by sedimentation (settling) or filtration. A variety of devices, such as baffles, static mixers, impellers and in-line sprays, can be used to mix the water and distribute the chemicals evenly.

CHEMICAL OXIDIZER: KMnO4 is used for taste and odor control because it is a strong oxidizer which eliminates many organic compounds.

CHEMICAL REATION RATE: In general, when the temperature decreases, the chemical reaction rate also decreases. The opposite is true for when the temperature increases.

CHEMICAL SLUDGE: Sludge resulting from chemical treatment processes of inorganic wastes that are not biologically active.

CHEMICAL OXYGEN DEMAND (COD): The milligrams of oxygen required to chemically oxidize the organic contaminants in one liter of wastewater.

CHLORAMINATION: Treating drinking water by applying chlorine before or after ammonia. This creates a persistent disinfectant residual called chloramines.

CHLORAMINES: A group of chlorine ammonia compounds formed when chlorine combines with organic wastes in the water. Chloramines are not effective as disinfectants and are responsible for eye and skin irritation as well as strong chlorine odors (also known as Combined Chlorine).

CHLORINATION: The process in water treatment of adding chlorine (gas or solid hypochlorite) for purposes of disinfection.

CHLORINE: A chemical used to disinfect water. Chlorine is extremely reactive, and when it comes in contact with microorganisms in water it kills them. Chlorine is added to swimming pools to keep the water safe for swimming. Chlorine is available as solid tablets for swimming pools. Some public water system's drinking water treatment plants use chlorine in a gas form because of the large volumes required. Chlorine is very effective against algae, bacteria and viruses. Protozoa are resistant to chlorine because they have thick coats; protozoa are removed from drinking water by filtration.

CHLORINE DEMAND: Amount of chlorine required to react on various water impurities before a residual is obtained. Also, means the amount of chlorine required to produce a free chlorine residual of 0.1 mg/l after a contact time of fifteen minutes as measured by iodmetic method of a sample at a temperature of twenty degrees in conformance with Standard methods.

CHLORINE FEED: Chlorine may be delivered by vacuum-controlled solution feed chlorinators. The chlorine gas is controlled, metered, introduced into a stream of injector water and then conducted as a solution to the point of application.

CHLORINE, FREE: Chlorine available to kill bacteria or algae. The amount of chlorine available for sanitization after the chlorine demand has been met. Also known as chlorine residual.

CIRCULATION: The continual flow of drilling fluid from injection to recovery and recirculation at the surface.

ClO_2: The molecular formula of Chlorine dioxide.

CLARIFIER: A settling tank used to remove suspended solids by gravity settling. Commonly referred to as sedimentation or settling basins, they are usually equipped with a motor driven chain and flight or rake mechanism to collect settled sludge and move it to a final removal point.

COMPOSITE SAMPLE: To have significant meaning, samples for laboratory tests on wastewater should be representative of the wastewater. The best method of sampling is proportional composite sampling over several hours during the day. Composite samples are collected because the flow and characteristics of the wastewater are continually changing. A composite sample will give a representative analysis of the wastewater conditions.

COAGULATION: The best pH range for coagulation is between 5 and 7. Mixing is an important part of the coagulation process you want to complete the coagulation process as quickly as possible. A chemical added to initially destabilize, aggregate, and bind together colloids and emulsions to improve settleability, filterability, or drainability.

COLIFORM: Bacteria normally found in the intestines of warm-blooded animals. Coliform bacteria are present in high numbers in animal feces. They are an indicator of potential contamination of water. Adequate and appropriate disinfection effectively destroys coliform bacteria. Public water systems are required to deliver safe and reliable drinking water to their customers 24 hours a day, 365 days a year. If the water supply becomes contaminated, consumers can become seriously ill. Fortunately, public water systems take many steps to ensure that the public has safe, reliable drinking water. One of the most important steps is to regularly test the water for coliform bacteria. Coliform bacteria are organisms that are present in the environment and in the feces of all warm-blooded animals and humans. Coliform bacteria will not likely cause illness. However, their presence in drinking water indicates that disease-causing organisms (pathogens) could be in the water system. Most pathogens that can contaminate water supplies come from the feces of humans or animals. Testing drinking water for all possible pathogens is complex, time-consuming, and expensive. It is relatively easy and inexpensive to test for coliform bacteria. If coliform bacteria are found in a water sample, water system operators work to find the source of contamination and restore safe drinking water. There are three different groups of coliform bacteria; each has a different level of risk.

COLIFORM TESTING: The effectiveness of disinfection is usually determined by Coliform bacteria testing. A positive sample is a bad thing and indicates that you have bacteria contamination.

COLLOIDAL SUSPENSIONS: Because both iron and manganese react with dissolved oxygen to form insoluble compounds, they are not found in high concentrations in waters containing dissolved oxygen except as colloidal suspensions of the oxide.

COLORIMETRIC MEASUREMENT: A means of measuring an unknown chemical concentration in water by measuring a sample's color intensity.

CHRONIC: A stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. Chronic should be considered a relative term depending on the life span of an organism. The measurement of chronic effect can be reduced growth, reduced reproduction, etc., in addition to lethality.

COMBINED CHLORINE: The reaction product of chlorine with ammonia or other pollutants, also known as chloramines.

COMPOSITE SAMPLE: A water sample that is a combination of a group of samples collected at various intervals during the day. A combination of individual samples of water or wastewater taken at predetermined intervals to minimize the effect of variability of individual samples.

COMPOSTING: Stabilization process relying on the aerobic decomposition of organic matter in sludge by bacteria and fungi.

CONDENSATION: The process that changes water vapor to tiny droplets or ice crystals.

CONTACT STABILIZATION PROCESS: Modification of the activated sludge process where raw wastewater is aerated with activated sludge for a short time prior to solids removal and continued aeration in a stabilization tank.

CONTACT TIME: If the water temperature decreases from 70°F (21°C) to 40°F (4°C). The operator needs to increase the detention time to maintain good disinfection of the water.

CONTAINS THE ELEMENT CARBON: A simple definition of an organic compound.

CONTAMINANT: Any natural or man-made physical, chemical, biological, or radiological substance or matter in water, which is at a level that may have an adverse effect on public health, and which is known or anticipated to occur in public water systems.

CONTAMINATE:
1. To make impure or unclean by contact or mixture.
2. To expose to or permeate with radioactivity.

CONTAMINATION: A degradation in the quality of groundwater in result of the it's becoming polluted with unnatural or previously non-existent constituents.

COPPER: The chemical name for the symbol Cu.

CORROSION: The removal of metal from copper, other metal surfaces and concrete surfaces in a destructive manner. Corrosion is caused by improperly balanced water or excessive water velocity through piping or heat exchangers.

CORROSIVITY: The Langelier Index measures corrosivity.

CROSS-CONNECTION: A physical connection between a public water system and any source of water or other substance that may lead to contamination of the water provided by the public water system through backflow. Might be the source of an organic substance causing taste and odor problems in a water distribution system.

CROSS-CONTAMINATION: The mixing of two unlike qualities of water. For example, the mixing of good water with a polluting substance like a chemical.

CRYPTOSPORIDIUM: A disease-causing parasite, resistant to chlorine disinfection. It may be found in fecal matter or contaminated drinking water. Cryptosporidium is a protozoan pathogen of the Phylum Apicomplexa and causes a diarrheal illness called cryptosporidiosis. Other apicomplexan pathogens include the malaria parasite Plasmodium, and Toxoplasma, the causative agent of toxoplasmosis. Unlike Plasmodium, which transmits via a mosquito vector, Cryptosporidium does not utilize an insect vector and is capable of completing its life cycle within a single host, resulting in cyst stages which are excreted in feces and are capable of transmission to a new host.

CYANURIC ACID: Chemical used to prevent the decomposition of chlorine by ultraviolet (UV) light.

CYANOBACTERIA: Cyanobacteria, also known as blue-green algae, blue-green bacteria or Cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis. The name "cyanobacteria" comes from the color of the bacteria (Greek: kyanós = blue). They are a significant component of the marine nitrogen cycle and an important primary producer in many areas of the ocean, but are also found on land.

CYST: A phase or a form of an organism produced either in response to environmental conditions or as a normal part of the life cycle of the organism. It is characterized by a thick and environmentally resistant cell wall.

DAILY MAXIMUM LIMITATIONS: The maximum allowable discharge of pollutants during a 24 hour period. Where daily maximum limitations are expressed in units of mass, the daily discharge is the total mass discharged over the course of the day. Where daily maximum limitations are expressed in terms of a concentration, the daily discharge is the arithmetic average measurement of the pollutant concentration derived from all measurements taken that day.

DANGEROUS CHEMICALS: The most suitable protection when working with a chemical that produces dangerous fumes is to work under an air hood.

DECANT: Separation of a liquid from settled solids by removing the upper layer of liquid after the solids have settled.

DECIBELS: The unit of measurement for sound.

DECOMPOSE: To decay or rot.

DECOMPOSTION OF ORGANIC MATERIAL: The decomposition of organic material in water produces taste and odors.

DEMINERALIZATION PROCESS: Mineral concentration of the feed water is the most important consideration in the selection of a demineralization process. Acid feed is the most common method of scale control in a membrane demineralization treatment system.

DENITRIFICATION: A biological process by which nitrate is converted to nitrogen gas.

DEPOLARIZATION: The removal of hydrogen from a cathode.

DESICCANT: When shutting down equipment which may be damaged by moisture, the unit may be protected by sealing it in a tight container. This container should contain a desiccant.

DESORPTION: Desorption is a phenomenon whereby a substance is released from or through a surface. The process is the opposite of sorption (that is, adsorption and absorption). This occurs in a system being in the state of sorption equilibrium between bulk phase (fluid, i.e. gas or liquid solution) and an adsorbing surface (solid or boundary separating two fluids). When the concentration (or pressure) of substance in the bulk phase is lowered, some of the sorbed substance changes to the bulk state. In chemistry, especially chromatography, desorption is the ability for a chemical to move with the mobile phase. The more a chemical desorbs, the less likely it will adsorb, thus instead of sticking to the stationary phase, the chemical moves up with the solvent front. In chemical separation processes, stripping is also referred to as desorption as one component of a liquid stream moves by mass transfer into a vapor phase through the liquid-vapor interface.

DIATOMACEOUS EARTH: A fine silica material containing the skeletal remains of algae.

DIGESTER: A tank or vessel used for sludge digestion.

DIGESTION: The biological decomposition of organic matter in sludge resulting in partial gasification, liquefaction, and mineralization of putrescible and offensive solids.

DIRECT CURRENT: A source of direct current (**DC**) may be used for standby lighting in a water treatment facility. The electrical current used in a DC system may come from a battery.

DISINFECT: The application of a chemical to kill most, but not all, microorganisms that may be present. Chlorine is added to public water drinking systems drinking water for disinfection. Depending on your state rule, drinking water must contain a minimum of 0.2 mg/L free chlorine. Disinfection makes drinking water safe to consume from the standpoint of killing pathogenic microorganisms including bacteria and viruses. Disinfection does not remove all bacteria from drinking water, but the bacteria that can survive disinfection with chlorine are not pathogenic bacteria that can cause disease in normal healthy humans.

DISINFECTION: The treatment of water to inactivate, destroy, and/or remove pathogenic bacteria, viruses, protozoa, and other parasites.

DISSOLVED OXYGEN: Can be added to zones within a lake or reservoir that would normally become anaerobic during periods of thermal stratification.

DISSOLVED SOLIDS: Solids in solution that cannot be removed by filtration with a 0.45 micron filter.

DISTILLATION, REVERSE OSMOSIS AND FREEZING: Processes that can be used to remove minerals from the water.

DRY ACID: A granular chemical used to lower pH and or total alkalinity.

E. COLI, Escherichia coli: A bacterium commonly found in the human intestine. For water quality analyses purposes, it is considered an indicator organism. These are considered evidence of water contamination. Indicator organisms may be accompanied by pathogens, but do not necessarily cause disease themselves.

ECDYSONE: A steroid hormone that triggers molting in arthropods.

ECOLOGICAL EFFICIENCY: The ratio of net productivity at one trophic level to net productivity at the next lower level.

ECOLOGICAL NICHE: The sum total of an organism's utilization of the biotic and abiotic resources of its environment. The fundamental niche represents the theoretical capabilities and the realized niche represents the actual role.

ECOLOGY: The study of how organisms interact with their environments.

ECOSYSTEM: The sum of physical features and organisms occurring in a given area.

ECTODERM: The outermost tissue layer of an animal embryo. Also, tissue derived from an embryonic ectoderm.

ECTOTHERM: An organism that uses environmental heat and behavior to regulate its body temperature.

EDWARD JENNER: A pioneer of vaccination; used vaccination with material from cowpox lesions to protect people against smallpox.

EFFECTIVENESS OF CHLORINE: The factors which influence the effectiveness of chlorination the most are pH, turbidity and temperature. Effectiveness of Chlorine decreases occurs during disinfection in source water with excessive turbidity.

EFFECTOR: The part of an organism that produces a response to a stimulus.

EFFLUENT: Partially or completely treated water or wastewater flowing out of a basin or treatment plant.

ELECTRICAL SYNAPSE: A junction between two neurons separated only by a gap junction, in which the local currents sparking the action potential pass directly between the cells.

ELECTROCARDIOGRAM: A plot of electrical activity of the heart over the cardiac cycle; measured via multiple skin electrodes.

ELECTROCHEMICAL GRADIENT: Combined electrostatic and osmotic-concentration gradient, such as the chemiosmotic gradient of mitochondria and chloroplasts.

ELECTROGENIC PUMP: An ion transport protein generating voltage across a membrane.

ELECTROMAGNETIC SPECTRUM: The entire spectrum of radiation; ranges in wavelength from less than a nanometer to more than a kilometer.

ELECTRON: The name of a negatively charged atomic particle. A negatively charged subatomic particle of an atom or ion. In atoms, the number of electrons present is equal to the number of positively charged protons present. Hence, atoms are electrically neutral.

ELECTRON MICROSCOPE: A microscope that focuses an electron beam through a specimen, resulting in resolving power a thousandfold greater than that of a light microscope. A transmission EM is used to study the internal structure of thin sections of cells; a scanning EM is used to study the ultrastructure of surfaces.

ELECTRON TRANSPORT CHAIN: A series of enzymes found in the inner membranes of mitochondria and chloroplasts. These are involved in transport of protons and electrons either across the membrane during ATP synthesis.

ELECTRONEGATIVITY: A property exhibited by some atoms whereby the nucleus has a tendency to pull electrons toward itself.

ELECTRONIC CHARGE UNIT: The charge of one electron (1.6021 x 10e - 19 coulomb).

ELECTROSTATIC FORCE: The attraction between particles with opposite charges.

ELECTROSTATIC GRADIENT: The free-energy gradient created by a difference in charge between two points, generally the two sides of a membrane.

ELEMENT: Any substance that cannot be broken down into another substance by ordinary chemical means.

ELIMINATION: The release of unabsorbed wastes from the digestive tract.

EMERGENT PROGERTY: A property exhibited at one level of biological organization but not exhibited at a lower level. For example, a population exhibits a birth rate, an organism does not.

EMULSION: A suspension, usually as fine droplets of one liquid in another. A mixture made up of dissimilar elements, usually of two or more mutually insoluble liquids that would normally separate into layers based on the specific gravity of each liquid.

ENDERGONIC: A phenomenon which involves uptake of energy.

ENDOCRINE: A phenomenon which relates to the presence of ductless glands of the type typically found in vertebrates. The endocrine system involves hormones, the glands which secrete them, the molecular hormone receptors of target cells, and interactions between hormones and the nervous system.

ENDOCYTOSIS: A process by which liquids or solid particles are taken up by a cell through invagination of the plasma membrane.

ENDODERM: The innermost germ layer of an animal embryo.

ENDODERMIS: A plant tissue, especially prominent in roots, that surrounds the vascular cylinder; all endodermal cells have Casparian strips.

ENDOMEMBRANE SYSTEM: The system of membranes inside a eukaryotic cell, including the membranous vesicles which associate with membrane sheets and/or tubes.

ENDOMETRIUM: The inner lining of the uterus, which is richly supplied with blood vessels that provide the maternal part of the placenta and nourish the developing embryo.

ENDONUCLEASE: An enzyme that breaks bonds within nucleic acids. A restriction endonuclease is an enzyme that breaks bonds only within a specific sequence of bases.

ENDOPLASMIC RETICULUM: A system of membrane-bounded tubes and flattened sacs, often continuous with the nuclear envelope, found in the cytoplasm of eukaryotes. Exists as rough ER, studded with ribosomes, and smooth ER, lacking ribosomes.

ENDORPHIN: A hormone produced in the brain and anterior pituitary that inhibits pain perception.

ENDOSKELETON: An internal skeleton.

ENDOSPERM: A nutritive material in plant seeds which is triploid (3n) and results from the fusion of three nuclei during double fertilization.

ENDOSYMBIOTIC: 1) An association in which the symbiont lives within the host 2) A widely accepted hypothesis concerning the evolution of the eukaryotic cell: the idea that eukaryotes evolved as a result of symbiotic associations between prokaryote cells. Aerobic symbionts ultimately evolved into mitochondria; photosynthetic symbionts became chloroplasts.

ENDOTHELIUM: The innermost, simple squamous layer of cells lining the blood vessels; the only constituent structure of capillaries.

ENDOTHERMIC: In chemistry, a phenomenon in which energy is absorbed by the reactants. In physiology, this term concerns organisms whose thermal relationship with the environment is dependent substantially on internal production of heat.

ENDOTOXIN: A component of the outer membranes of certain gram-negative bacteria responsible for generalized symptoms of fever and ache.

ENERGY: The capacity to do work by moving matter against an opposing force.

ENHANCER: A DNA sequence that recognizes certain transcription factors that can stimulate transcription of nearby genes.

ENTAMOEBA HISTOLYTICA: Entamoeba histolytica, another water-borne pathogen, can cause diarrhea or a more serious invasive liver abscess. When in contact with human cells, these amoebae are cytotoxic. There is a rapid influx of calcium into the contacted cell, it quickly stops all membrane movement save for some surface blebbing. Internal organization is disrupted, organelles lyse, and the cell dies. The ameba may eat the dead cell or just absorb nutrients released from the cell.

ENTERIC: Rod-shaped, gram-negative, aerobic but can live in certain anaerobic conditions; produce nitrite from nitrate, acids from glucose; include Escherichia coli, Salmonella (over 1000 types), and Shigella.

ENTEROVIRUS: A virus whose presence may indicate contaminated water; a virus that may infect the gastrointestinal tract of humans.

ENTROPY: A type of energy which is not biologically useful to do work (in contrast to free energy).

ENVELOPE: 1) (nuclear) The surface, consisting of two layers of membrane, that encloses the nucleus of eukaryotic cells. 2) (virus) A structure which is present on the outside of some viruses (exterior to the capsid).

ENVIRONMENT: Water, air, and land, and the interrelationship that exists among and between water, air and land and all living things. The total living and nonliving aspects of an organism's internal and external surroundings.

ENZYME: A protein, on the surface of which are chemical groups so arranged as to make the enzyme a catalyst for a chemical reaction.

EPICOTYL: A portion of the axis of a plant embryo above the point of attachment of the cotyledons; forms most of the shoot.

EPIDERMIS: The outermost portion of the skin or body wall of an animal.

EPINEPHRINE: A hormone produced as a response to stress; also called adrenaline.

EPIPHYTE: A plant that nourishes itself but grows on the surface of another plant for support, usually on the branches or trunks of tropical trees.

EPISOME: Genetic element at times free in the cytoplasm, at other times integrated into a chromosome.

EPISTASIS: A phenomenon in which one gene alters the expression of another gene that is independently inherited.

EPITHELIUM: An animal tissue that forms the covering or lining of all free body surfaces, both external and internal.

EPITOPE: A localized region on the surface of an antigen that is chemically recognized by antibodies; also called antigenic determinant.

EQUATION: A precise representation of the outcome of a chemical reaction, showing the reactants and products, as well as the proportions of each.

EQUILIBRIUM: In a reversible reaction, the point at which the rate of the forward reaction equals that of the reverse reaction. (constant) At equilibrium, the ratio of products to reactants. (potential) The membrane potential for a given ion at which the voltage exactly balances the chemical diffusion gradient for that ion.

ERNST MAYR: Formulated the biological species concept.

ERYTHROCYTE: A red blood corpuscle.

ESOPHAGUS: An anterior part of the digestive tract; in mammals it leads from the pharynx to the stomach.

ESSENTIAL: 1) An amino or fatty acid which is required in the diet of an animal because it cannot be synthesized. 2) A chemical element required for a plant to grow from a seed and complete the life cycle.

ESTIVATION: A physiological state characterized by slow metabolism and inactivity, which permits survival during long periods of elevated temperature and diminished water supplies.

ESTRADIOL: 1,3,5(10)-estratriene- 3,17 beta-diol C18H24O2. This is the natural hormone - present in pure form in the urine of pregnant mares and in the ovaries of pigs.

ESTROGEN: Any of a group of vertebrate female sex hormones.

ESTROUS CYCLE: In female mammals, the higher primates excepted, a recurrent series of physiological and behavioral changes connected with reproduction.

ESTRUS: The limited period of heat or sexual receptivity that occurs around ovulation in female mammals having estrous cycles.

ESTUARY: That portion of a river that is close enough to the sea to be influenced by marine tides.

ETHYLENE: The only gaseous plant hormone, responsible for fruit ripening, growth inhibition, leaf abscission, and aging.

EUBACTERIA: The lineage of prokaryotes that includes the cyanobacteria and all other contemporary bacteria except archaebacteria.

EUCHROMATIN: The more open, unraveled form of eukaryotic chromatin, which is available for transcription.

EUCOELOMATE: An animal whose body cavity is completely lined by mesoderm, the layers of which connect dorsally and ventrally to form mesenteries.

EUGLENA: Euglena are common protists, of the class Euglenoidea of the phylum Euglenophyta. Currently, over 1000 species of Euglena have been described. Marin et al. (2003) revised the genus so and including several species without chloroplasts, formerly classified as Astasia and Khawkinea. Euglena sometimes can be considered to have both plant and animal features. Euglena gracilis has a long hair-like thing that stretches from its body. You need a very powerful microscope to see it. This is called a flagellum, and the euglena uses it to swim. It also has a red eyespot. Euglena gracilis uses its eyespot to locate light. Without light, it cannot use its chloroplasts to make itself food.

EUKARYOTE: A life form comprised of one or more cells containing a nucleus and membrane - bound organelles. Included are members of the Kingdoms Protista, Fungi, Plantae and Animalia.

EUMETAZOA: Members of the subkingdom that includes all animals except sponges.

EUTROPHIC: A highly productive condition in aquatic environments which owes to excessive concentrations of nutrients which support the growth of primary producers.

EVAGINATED: Folded or protruding outward.

EVAPORATIVE COOLING: The property of a liquid whereby the surface becomes cooler during evaporation, owing to the loss of highly kinetic molecules to the gaseous state.

EVERSIBLE: Capable of being turned inside out.

EVOLUTION: A theory that all of the changes that have transformed life on earth from its earliest beginnings to the diversity that characterizes it today. As used in biology, the term evolution means descent with change. See Intelligent Design.

EXCITABLE CELLS: A cell, such as a neuron or a muscle cell that can use changes in its membrane potential to conduct signals.

EXCITATORY POSTSYNAPTIC POTENTIAL: An electrical change (depolarization) in the membrane of a postsynaptic neuron caused by the binding of an excitatory neurotransmitter from a presynaptic cell to a postsynaptic receptor. This phenomenon facilitates generation of an action potential in the PSP.

EXCRETION: Release of materials which arise in the body due to metabolism (e.g., CO_2, NH_3, H_20).

EXERGONIC: A phenomenon which involves the release of energy.

EXOCYTOSIS: A process by which a vesicle within a cell fuses with the plasma membrane and releases its contents to the outside.

EXON: A part of a primary transcript (and the corresponding part of a gene) that is ultimately either translated (in the case of mRNA) or utilized in a final product, such as tRNA.

EXOSKELETON: An external skeleton, characteristic of members of the phylum, Arthropoda.

EXOTHERMIC: A process or reaction that is accompanied by the creation of heat.

EXOTOXIN: A toxic protein secreted by a bacterial cell that produces specific symptoms even in the absence of the bacterium.

EXPONENTIAL: (population growth) The geometric increase of a population as it grows in an ideal, unlimited environment.

EXTRAEMBRYONIC MEMBRANES: Four membranes (yolk sac, amnion, chorion, allantois) that support the developing embryo in reptiles, birds, and mammals.

EXTRINSIC: External to, not a basic part of; as in extrinsic isolating mechanism.

F: The chemical symbol of Fluorine.

F PLASMID: The fertility factor in bacteria, a plasmid that confers the ability to form pili for conjugation and associated functions required for transfer of DNA from donor to recipient.

F1 GENERATION: The first filial or hybrid offspring in a genetic cross-fertilization.

F2 GENERATION: Offspring resulting from interbreeding of the hybrid F1 generation.

FACILITATED DIFFUSION: Passive movement through a membrane involving a specific carrier protein; does not proceed against a concentration gradient.

FACULTATIVE: An organism which exhibits the capability of changing from one habit or metabolic pathway to another, when conditions warrant. (anaerobe) An organism that makes ATP by aerobic respiration if oxygen is present but that switches to fermentation under anaerobic conditions.

FAT: A biological compound consisting of three fatty acids linked to one glycerol molecule.

FATE MAP: A means of tracing the fates of cells during embryonic development.

FATTY ACID: A long carbon chain carboxylic acid. Fatty acids vary in length and in the number and location of double bonds; three fatty acids linked to a glycerol molecule form fat.

FAUNA: The animals of a given area or period.

FEATURE DETECTOR: A circuit in the nervous system that responds to a specific type of feature, such as a vertically moving spot or a particular auditory time delay.

FECAL COLIFORM: A group of bacteria that may indicate the presence of human or animal fecal matter in water. Total coliform, fecal coliform, and E. coli are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. E. coli is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or E. coli, depending on the lab testing method.

FECES: Indigestible wastes discharged from the digestive tract.

FEEDBACK: The process by which a control mechanism is regulated through the very effects it brings about. Positive feedback is when the effect is amplified; negative feedback is when the effect tends toward restoration of the original condition. Feedback inhibition is a method of metabolic control in which the end-product of a metabolic pathway acts as an inhibitor of an enzyme within that pathway.

FERMENTATION: Anaerobic production of alcohol, lactic acid or similar compounds from carbohydrate resulting from glycolysis.

FERRIC CHLORIDE: An iron salt commonly used as a coagulant. Chemical formula is FeCl3.

FIBRIN: The activated form of the blood: clotting protein fibrinogen, which aggregates into threads that form the fabric of the clot.

FIBROBLAST: A type of cell in loose connective tissue that secretes the protein ingredients of the extracellular fibers.

FIBRONECTINS: A family of extracellular glycoproteins that helps embryonic cells adhere to their substrate as they migrate.

FILTER: A device utilizing a granular material, woven cloth or other medium to remove pollutants from water, wastewater or air.

FILTER AID: A polymer or other material added to improve the effectiveness of the filtration process.

FILTER CAKE: The layer of solids that is retained on the surface of a filter.

FILTER CLOGGING: An inability to meet demand may occur when filters are clogging.

FILTER PRESS: A dewatering device where sludge is pumped onto a filtering medium and water is forced out of the sludge, resulting in a “cake”.

FILTRATE: Liquid remaining after removal of solids with filtration.

FILTRATION RATE: A measurement of the volume of water applied to a filter per unit of surface area in a given period of time.

FITNESS: The extent to which an individual passes on its genes to the next generation. Relative fitness is the number of offspring of an individual compared to the mean.

FIXATION: 1) Conversion of a substance into a biologically more usable form, for example, CO_2 fixation during photosynthesis and N_2 fixation. 2) Process of treating living tissue for microscopic examination.

FIXED ACTION PATTERN (FAP): A highly: stereotyped behavior that is innate and must be carried to completion once initiated.

FLACCID: Limp; walled cells are flaccid in isotonic surroundings, where there is no tendency for water to enter.

FLAGELLIN: The protein from which prokaryotic flagella are constructed.

FLAGELLUM: A long whip-like appendage that propels cells during locomotion in liquid solutions. The prokaryote flagellum is comprised of a protein, flagellin. The eukaryote flagellum is longer than a cilium, but as a similar internal structure of microtubules in a"9 + 2" arrangement.

FLAME CELL: A flagellated cell associated with the simplest tubular excretory system, present in flatworms: it acts to directly regulate the contents of the extracellular fluid.

FLOCCULATION: The process of bringing together destabilized or coagulated particles to form larger masses that can be settled and/or filtered out of the water being treated. Conventional coagulation–flocculation-sedimentation practices are essential pretreatments for many water purification systems—especially filtration treatments. These processes agglomerate suspended solids together into larger bodies so that physical filtration processes can more easily remove them. Particulate removal by these methods makes later filtering processes far more effective. The process is often followed by gravity separation (sedimentation or flotation) and is always followed by filtration. A chemical coagulant, such as iron salts, aluminum salts, or polymers, is added to source water to facilitate bonding among particulates. Coagulants work by creating a chemical reaction and eliminating the negative charges that cause particles to repel each other. The coagulant-source water mixture is then slowly stirred in a process known as flocculation. This water churning induces particles to collide and clump together into larger and more easily removable clots, or “flocs.” The process requires chemical knowledge of source water characteristics to ensure that an effective coagulant mix is employed. Improper coagulants make these treatment methods ineffective. The ultimate effectiveness of coagulation/flocculation is also determined by the efficiency of the filtering process with which it is paired.

FLOCCULANTS: Flocculants, or flocculating agents, are chemicals that promote flocculation by causing colloids and other suspended particles in liquids to aggregate, forming a floc. Flocculants are used in water treatment processes to improve the sedimentation or filterability of small particles. For example, a flocculant may be used in swimming pool or drinking water filtration to aid removal of microscopic particles which would otherwise cause the water to be cloudy and which would be difficult or impossible to remove by filtration alone. Many flocculants are multivalent cations such as aluminum, iron, calcium or magnesium. These positively charged molecules interact with negatively charged particles and molecules to reduce the barriers to aggregation. In addition, many of these chemicals, under appropriate pH and other conditions such as temperature and salinity, react with water to form insoluble hydroxides which, upon precipitating, link together to form long chains or meshes, physically trapping small particles into the larger floc. Long-chain polymer flocculants, such as modified polyacrylamides, are manufactured and sold by the flocculant producing business. These can be supplied in dry or liquid form for use in the flocculation process. The most common liquid polyacrylamide is supplied as an emulsion with 10-40 % actives and the rest is a carrier fluid, surfactants and latex. Emulsion polymers require activation to invert the emulsion and allow the electrolyte groups to be exposed.
The following chemicals are used as flocculants:
* alum
* aluminum chlorohydrate
* aluminum sulfate
* calcium oxide
* iron(III) chloride
* iron(II) sulfate
* polyacrylamide
* sodium aluminate
* sodium silicate

FLOC SHEARING: Likely to happen to large floc particles when they reach the flocculation process.

FLOCCULATION BASIN: A compartmentalized basin with a reduction of speed in each compartment. This set-up or basin will give the best overall results.

FLOOD RIM: The point of an object where the water would run over the edge of something and begin to cause a flood.

FLORA: The plants of a given area or period.

FLOW CYTOMETER: A particle-sorting instrument capable of counting protozoa.

FLOW MUST BE MEASURED: A recorder that measures flow is most likely to be located in a central location.

FLUID FEEDER: An animal that lives by sucking nutrient-rich fluids from another living organism.

FLUID MOSAIC MODEL: The currently accepted model of cell membrane structure, which envisions the membrane as a mosaic of individually inserted protein molecules drifting laterally in a fluid bilayer of phospholipids.

FLUX: The term flux describes the rate of water flow through a semipermeable membrane. When the water flux decreases through a semipermeable membrane, it means that the mineral concentration of the water is increasing.

FLY ASH: The noncombustible particles in flue gas. Often used as a body feed or solidification chemical.

FOLLICLE STIMULATING HORMONE (FSH): A gonadotropic hormone of the anterior pituitary that stimulates growth of follicles in the ovaries of females and function of the seminiferous tubules in males.

FOLLICLE: A jacket of cells around an egg cell in an ovary.

FOOD CHAIN: Sequence of organisms, including producers, consumers, and decomposers, through which energy and materials may move in a community.

FOOD WEB: The elaborate, interconnected feeding relationships in an ecosystem.

FOOT CANDLE: Unit of illumination; the illumination of a surface produced by one standard candle at a distance of one foot.

FORMULA: A precise representation of the structure of a molecule or ion, showing the proportion of atoms which comprise the material.

FOUNDER EFFECT: The difference between the gene pool of a population as a whole and that of a newly isolated population of the same species.

FRACTIONATION: An experimental technique which involves separation of parts of living tissue from one another using centrifugation.

FRAGMENTATION: A mechanism of asexual reproduction in which the parent plant or animal separates into parts that reform whole organisms.

FRAMESHIFT MUTATION: A mutation occurring when the number of nucleotides inserted or deleted is not a multiple of 3, thus resulting in improper grouping into codons.

FREE CHLORINE: In disinfection, chlorine is used in the form of free chlorine or as hypochlorite ion.

FREE CHLORINE RESIDUAL: Regardless of whether pre-chloration is practiced or not, a free chlorine residual of at least 10 mg/L should be maintained in the clear well or distribution reservoir immediately downstream from the point of post-chlorination. The reason for chlorinating past the breakpoint is to provide protection in case of backflow.

FREE ENERGY OF ACTIVATION: See Activation energy.

FREE ENERGY: Usable energy in a chemical system; energy available for producing change.

FREE OIL: Non-emulsified oil that separates from water, in a given period of time.

FREQUENCY DEPENDENT SELECTION: A decline in the reproductive success of a morph resulting from the morph's phenotype becoming too common in a population; a cause of balanced polymorphism in populations.

FUNCTIONAL GROUP: One of several groups of atoms commonly found in organic molecules. A functional group contributes somewhat predictable properties to the molecules which possess them.

FUNDAMENTAL NICHE: The total resources an organism is theoretically capable of utilizing.

G: (protein) A membrane protein that serves as an intermediary between hormone receptors and the enzyme adenylate cyclase, which converts ATP to cAMP in the second messenger system in non-steroid hormone action. Depending on the system, G proteins either increase or decrease cAMP production.

G1 PHASE: The first growth phase of the cell cycle, consisting of the portion of interphase before DNA synthesis is initiated.

G2 PHASE: The second growth phase of the cell cycle, consisting of the portion of interphase after DNA synthesis but before mitosis.

GAIA HYPOTHESIS: An idea, first formulated by James E. Lovelock in 1979, which suggests that the biosphere of the earth exists as a "superorganism" which exhibits homeostatic self- regulation of the environment-biota global system.

GAMETANGIUM: The reproductive organ of bryophytes, consisting of the male antheridium and female archegonium; a multi-chambered jacket of sterile cells in which gametes are formed.

GAMETE: A sexual reproductive cell that must usually fuse with another such cell before development begins; an egg or sperm.

GAMETOPHYTE: A haploid plant that can produce gametes.

GANGLION: A structure containing a group of cell bodies of neurons.

GAP JUNCTION: A narrow gap between plasma membranes of two animal cells, spanned by protein channels. They allow chemical substances or electrical signals to pass from cell to cell.

GASTRULA: A two-layered, later three-layered, animal embryonic stage.

GASTRULATION: The process by which a blastula develops into a gastrula, usually by an involution of cells.

GATED ION CHANNEL: A membrane channel that can open or close in response to a signal, generally a change in the electrostatic gradient or the binding of a hormone, transmitter, or other molecular signal.

GEL ELECTROPHORESIS: In general, electrophoresis is a laboratory technique used to separate macromolecules on the basis of electric charge and size; the technique involves application of an electric field to a population of macromolecules which disperse according to their electric mobilities. In gel electrophoresis, the porous medium through which the macromolecules move is a gel.

GEL: Colloid in which the suspended particles form a relatively orderly arrangement.

GENE: The hereditary determinant of a specified characteristic of an individual; specific sequences of nucleotides in DNA.

GENE AMPLIFICATION: Any of the strategies that give rise to multiple copies of certain genes, thus facilitating the rapid synthesis of a product (such as rRna for ribosomes) for which the demand is great.

GENE CLONING: Formation by a bacterium, carrying foreign genes in a recombinant plasmid, of a clone of identical cells containing the replicated foreign genes.

GENE DELIVERY: This is a general term for the introduction of new genetic elements into the genomes of living cells. The delivery problem is essentially conditioned by the fact that the new genetic elements are usually large, and by the presence of the outer cell membrane and the nuclear membrane acting as barriers to incorporation of the new DNA into the genome already present in the nucleus. Viruses possess various natural biochemical methods for achieving gene delivery; artificial gene delivery is one of the essential problems of "genetic engineering". The most important barrier is apparently the outer cell membrane, which is essentially a lipid barrier, and introduction of any large complex into the cell requires a fusion of one kind or another with this membrane. Liposomes, which consist of lipid membranes themselves, and which can fuse with outer cell membranes, are thus potential vehicles for delivery of many substances, including DNA.

GENE FLOW: The movement of genes from one part of a population to another, or from one population to another, via gametes.

GENE POOL: The sum total of all the genes of all the individuals in a population.

GENE REGULATION: Any of the strategies by which the rate of expression of a gene can be regulated, as by controlling the rate of transcription.

GENETIC DRIFT: Change in the gene pool as a result of chance and not as a result of selection, mutation, or migration.

GENETIC RECOMBINATION: The general term for the production of offspring that combine traits of the two parents.

GENETICS: The science of heredity; the study of heritable information.

GENOME: The cell's total complement of DNA.

GENOMIC EQUIVALENCE: The presence of all of an organism's genes in all of its cells.

GENOMIC IMPRINTING: The parental effect on gene expression. Identical alleles may have different effects on offspring depending on whether they arrive in the zygote via the ovum or via the sperm.

GENOMIC LIBRARY: A set of thousands of DNA segments from a genome, each carried by a plasmid or phage.

GENOTYPE: The particular combination of genes present in the cells of an individual.

GENUS: A taxonomic category above the species level, designated by the first word of a species' binomial Latin name.

GIARDIA LAMLIA: Giardia lamblia (synonymous with Lamblia intestinalis and Giardia duodenalis) is a flagellated protozoan parasite that colonizes and reproduces in the small intestine, causing giardiasis. The giardia parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastro-intestinal tract, but remains confined to the lumen of the small intestine. Giardia trophozoites absorb their nutrients from the lumen of the small intestine, and are anaerobes.

GIARDIASAS, HEPATITIS OR TYHOID: Diseases that may be transmitted through the contamination of a water supply, but not AIDS.

GIS – GRAPHIC INFORMATION SYSTEM: Detailed information about the physical locations of structures such as pipes, valves, and manholes within geographic areas with the use of satellites.

GLIAL CELL: A non-conducting cell of the nervous system that provides support, insulation, and protection for the neurons.

GLIDING: Rod-shaped, gram-negative, mostly aerobic; glide on secreted slimy substances; form colonies, frequently with complex fruiting structures.

GLOMERULUS: A capillary bed within Bowman's capsule of the nephron; the site of ultrafiltration.

GLUCOSE: A six carbon sugar which plays a central role in cellular metabolism.

GLYCOCALYX: The layer of protein and carbohydrates just outside the plasma membrane of an animal cell; in general, the proteins are anchored in the membrane, and the carbohydrates are bound to the proteins.

GLYCOGEN: A long, branched polymer of glucose subunits that is stored in the muscles and liver of animals and is metabolized as a source of energy.

GLYCOLYSIS: A metabolic pathway which occurs in the cytoplasm of cells and during which glucose is oxidized anaerobically to form pyruvic acid.

GLYCOPROTEIN: A protein with covalently linked sugar residues. The sugars may be bound to OH side chains of the polypeptide (O: linked) or the amide nitrogen of asparagine side chains (N: linked).

GLYCOSIDIC: A type of bond which links monosaccharide subunits together in di- or polysaccharides.

GLYOXYSOME: A type of microbody found in plants, in which stored lipids are converted to carbohydrates.

GOLGI APPARATUS: A system of concentrically folded membranes found in the cytoplasm of eukaryotic cells. Plays a role in the production and release of secretory materials such as the digestive enzymes manufactured in the pancreas.

GONADOTROPIN: Refers to a member of a group of hormones capable of promoting growth and function of the gonads. Includes hormones such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) which are stimulatory to the gonads.

GOOD CONTACT TIME, pH and LOW TURBIDITY: These are factors that are important in providing good disinfection when using chlorine.

GPM: Gallons per minute.

GRAB SAMPLE: A sample which is taken from a water or wastestream on a one-time basis with no regard to the flow of the water or wastestream and without consideration of time. A single grab sample should be taken over a period of time not to exceed 15 minutes.

GRAB SAMPLE: A single water or wastewater sample taken at a time and place representative of total discharge.

GRADED POTENTIAL: A local voltage change in a neuron membrane induced by stimulation of a neuron, with strength proportional to the strength of the stimulus and lasting about a millisecond.

GRANUM: A stack-like grouping of photosynthetic membranes in a chloroplast

GRAVITY BELT THICKENER: A sludge dewatering device utilizing a filter belt to promote gravity drainage of water. Usually precedes additional dewatering treatment.

GRAVITY FILTER: A filter that operates at atmospheric pressure.

GRAVITY THICKENING: A sedimentation basin designed to operate at high solids loading rates.

GRAVITROPISM: A response of a plant or animal in response to gravity.

GREENHOUS EFFECT: The warming of the Earth due to atmospheric accumulation of carbon dioxide which absorbs infrared radiation and slows its escape from the irradiated Earth.

GREGOR MENDEL: The first to make quantitative observations of the patterns of inheritance and proposing plausible explanations for them.

GROWTH FACTOR: A protein that must be present in a cell's environment for its normal growth and development.

GT: Represents (Detention time) x (mixing intensity) in flocculation.

GUARD CELL: A specialized epidermal cell that regulates the size of stoma of a leaf.

GYMNOSPERM: A vascular plant that bears naked seeds not enclosed in any specialized chambers.

H2SO4: The molecular formula of Sulfuric acid.

HABIT: In biology, the characteristic form or mode of growth of an organism.

HABITAT: The kind of place where a given organism normally lives.

HABITUATION: The process that results in a long-lasting decline in the receptiveness of interneurons to the input from sensory neurons or other interneurons (sensitization, adaptation).

HALF: The average amount of time it takes for one-half of a specified quantity of a substance to decay or disappear.

HALIDES: A halide is a binary compound, of which one part is a halogen atom and the other part is an element or radical that is less electronegative than the halogen, to make a fluoride, chloride, bromide, iodide, or astatide compound. Many salts are halides. All Group 1 metals form halides with the halogens and they are white solids. A halide ion is a halogen atom bearing a negative charge. The halide anions are fluoride (F), chloride (Cl), bromide (Br), iodide (I) and astatide (At). Such ions are present in all ionic halide salts.

HALOACETIC ACIDS: Haloacetic acids are carboxylic acids in which a halogen atom takes the place of a hydrogen atom in acetic acid. Thus, in a monohaloacetic acid, a single halogen would replace a hydrogen atom. For example, chloroacetic acid would have the structural formula CH_2ClCO_2H. In the same manner, in dichloroacetic acid two chlorine atoms would take the place of two hydrogen atoms ($CHCl_2CO_2H$).

HAPLOID: The condition of having only one kind of a given type of chromosome.

HARD WATER: Hard water causes a buildup of scale in household hot water heaters. Hard water is a type of water that has high mineral content (in contrast with soft water). Hard water primarily consists of calcium (Ca2+), and magnesium (Mg2+) metal cations, and sometimes other dissolved compounds such as bicarbonates and sulfates. Calcium usually enters the water as either calcium carbonate ($CaCO_3$), in the form of limestone and chalk, or calcium sulfate ($CaSO_4$), in the form of other mineral deposits. The predominant source of magnesium is dolomite ($CaMg(CO_3)2$). Hard water is generally not harmful. The simplest way to determine the hardness of water is the lather/froth test: soap or toothpaste, when agitated, lathers easily in soft water but not in hard water. More exact measurements of hardness can be obtained through a wet titration. The total water 'hardness' (including both Ca2+ and Mg2+ ions) is read as parts per million or weight/volume (mg/L) of calcium carbonate (CaCO3) in the water. Although water hardness usually only measures the total concentrations of calcium and magnesium (the two most prevalent, divalent metal ions), iron, aluminum, and manganese may also be present at elevated levels in some geographical locations.

HARDNESS: A measure of the amount of calcium and magnesium salts in water. More calcium and magnesium lead to greater hardness. The term "hardness" comes from the fact that it is hard to get soap suds from soap or detergents in hard water. This happens because calcium and magnesium react strongly with negatively-charged chemicals like soap to form insoluble compounds.

HARDY-WEINBERG THEOREM: An axiom maintaining that the sexual shuffling of genes alone cannot alter the overall genetic makeup of a population.

HAUSTORIUM: In parasitic fungi, a nutrient-absorbing hyphal tip that penetrates the tissues of the host but remains outside the host cell membranes.

HAVERSIAN SYSTEM: One of many structural units of vertebrate bone, consisting of concentric layers of mineralize bone matrix surrounding lacunae, which contain osteocytes, and a central canal, which contains blood vessels and nerves.

HAZARDS OF POLYMERS: Slippery and difficult to clean-up are the most common hazards associated with the use of polymers in a water treatment plant.

HEAD: The measure of the pressure of water expressed in feet of height of water. 1 PSI = 2.31 feet of water or 1 foot of head equals about a half a pound of pressure or .433 PSI. There are various types of heads of water depending upon what is being measured. Static (water at rest) and Residual (water at flow conditions).

HEADWORKS: The facility at the "**head**" of the water source where water is first treated and routed into the distribution system.

HEALTH ADVISORY: An EPA document that provides guidance and information on contaminants that can affect human health and that may occur in drinking water, but which the EPA does not currently regulate in drinking water.

HEAT OF VAPORIZATION: The amount of energy absorbed by a substance when it changes state to a gas. Water absorbs approximately 580 calories per gram when it changes from liquid water to water vapor.

HEAT: The total amount of kinetic energy due to molecular motion in a body of matter. Heat is energy in its most random form.

HELPER T CELL: A type of T cell that is required by some B cells to help them make antibodies or that helps other T cells respond to antigens or secrete lymphokines or interleukins.

HEMAGGLUTININ: A surface antigen on influenza viruses which controls infectivity by associating with receptors on host erythrocytes or other cells.

HEMATOPOIESIS: The formation of blood.

HEMATOPOIETIC STEM CELLS: Cells found in the bone marrow of adult mammals which give rise to erythroid stem cells, lymphoid stem cells, and myeloid stem cells. Such cells give rise to erythrocytes and a variety of types of lymphocytes and leucocytes.

HEMOGLOBIN: An iron-containing respiratory pigment found in many organisms.

HEMOLYMPH: In invertebrates with open circulatory systems, the body fluid that bathes tissues.

HEMOPHILIA: A genetic disease resulting from an abnormal sex-linked recessive gene, characterized by excessive bleeding following injury.

HEPATIC: Pertaining to the liver.

HEREDITY: A biological phenomenon whereby characteristics are transmitted from one generation to another by virtue of chemicals (i.e. DNA) transferred during sexual or asexual reproduction.

HERPESVIRUS: A double stranded DNA virus with an enveloped, icosahedral capsid.

HERTZ: The term used to describe the frequency of cycles in an alternating current (AC) circuit. A unit of frequency equal to one cycle per second.

HETEROCHROMATIN: Non-transcribed eukaryotic chromatin that is so highly compacted that it is visible with a light microscope during interphase.

HETEROCHRONY: Evolutionary changes in the timing or rate of development.

HETEROCYST: A specialized cell that engages in nitrogen fixation on some filamentous cyanobacteria.

HETEROGAMY: The condition of producing gametes of two different types (contrast with isogamy).

HETEROMORPHIC: A condition in the life cycle of all modern plants in which the sporophyte and gametophyte generations differ in morphology.

HETEROSPOROUS: Referring to plants in which the sporophyte produces two kinds of spores that develop into unisexual gametophytes, either male or female.

HETEROTROPH: An organism dependent on external sources of organic compounds as a means of obtaining energy and/or materials. Such an organism requires carbon ("food") from its environment in an organic form. (synonym-organotroph).

HETEROTROPHIC PLATE COUNT: A test performed on drinking water to determine the total number of all types of bacteria in the water.

HETEROZYGOTE ADVANTAGE: A mechanism that preserves variation in eukaryotic gene pools by conferring greater reproductive success on heterozygotes over individuals homozygous for any one of the associated alleles.

HETEROZYGOUS: The condition whereby two different alleles of the gene are present within the same cell.

HF: The molecular formula of Hydrofluoric acid.

HIGH TURBIDITY CAUSING INCREASED CHLORINE DEMAND: May occur or be caused by the inadequate disinfection of water.

HISTAMINE: A substance released by injured cells that causes blood vessels to dilate during an inflammatory response.

HISTOLOGY: The study of tissues.

HISTONE: A type of protein characteristically associated with the chromosomes of eukaryotes.

HIV-1: Acute human immunodeficiency virus type 1 is the subtype of HIV (human immune deficiency virus) that causes most cases of AIDS in the Western Hemisphere, Europe, and Central, South, and East Africa. HIV is a retrovirus (subclass lentivirus), and retroviruses are single: stranded RNA viruses that have an enzyme called reverse transcriptase. With this enzyme the viral RNA is used as a template to produce viral DNA from cellular material. This DNA is then incorporated into the host cell's genome, where it codes for the synthesis of viral components. An HIV-1 infection should be distinguished from AIDS. Acquired immunodeficiency syndrome (AIDS) is a secondary immunodeficiency syndrome resulting from HIV infection and characterized by opportunistic infections, malignancies, neurologic dysfunction, and a variety of other syndromes.

HOLOBLASTIC: A type of cleavage in which there is complete division of the egg, as in eggs having little yolk (sea urchin) or a moderate amount of yolk (frog).

HOME RANGE: An area within which an animal tends to confine all or nearly all its activities for a long period of time.

HOMEOBOX: Specific sequences of DNA that regulate patterns of differentiation during development of an organism.

HOMEOSTASIS: A phenomenon whereby a state or process (for example, within an organism) is regulated automatically despite the tendency for fluctuations to occur.

HOMEOTHEMIC: Capable of regulation of constancy with respect to temperature.

HOMEOTIC GENES: Genes that control the overall body plan of animals by controlling the developmental fate of groups of cells.

HOMEOTIC: (mutation) A mutation in genes regulated by positional information that results in the abnormal substitution of one type of body part in place of another.

HOMOLOGOUS CHROMOSOMES: Chromosomes bearing genes for the same characters.

HOMOLOGOUS STRUCTURES: Characters in different species which were inherited from a common ancestor and thus share a similar ontogenetic pattern.

HOMOLOGY: Similarity in characteristics resulting from a shared ancestry.

HOMOPLASY: The presence in several species of a trait not present in their most common ancestor. Can result from convergent evolution, reverse evolution, or parallel evolution.

HOMOSPOROUS: Referring to plants in which a single type of spore develops into a bisexual gametophyte having both male and female sex organs.

HOMOZYGOUS: Having two copies of the same allele of a given gene.

HORMONE: A control chemical secreted in one part of the body that affects other parts of the body.

HOST RANGE: The limited number of host species, tissues, or cells that a parasite (including viruses and bacteria) can infect.

HUMORAL IMMUNITY: The type of immunity that fights bacteria and viruses in body fluids with antibodies that circulate in blood plasma and lymph, fluids formerly called humors.

HYBIRD VIGOR: Increased vitality (compared to that of either parent stock) in the hybrid offspring of two different, inbred parents.

HYBIRD: In evolutionary biology, a cross between two species. In genetics, a cross between two genetic types.

HYBIRDIZATION: The process whereby a hybrid results from interbreeding two species; 2) DNA hybridization is the comparison of whole genomes of two species by estimating the extent of hydrogen bonding that occurs between single-stranded DNA obtained from the two species.

HYBRIDOMA: A hybrid cell that produces monoclonal antibodies in culture, formed by the fusion of a myeloma cell with a normal antibody-producing lymphocyte.

HYDRATED LIME: The calcium hydroxide product that results from mixing quicklime with water. Chemical formula is CaOH2.

HYDRATION SHELL: A "covering" of water molecules which surrounds polar or charged substances in aqueous solutions. The association is due to the charged regions of the polar water molecules themselves.

HYDRIDES: Hydride is the name given to the negative ion of hydrogen, H. Although this ion does not exist except in extraordinary conditions, the term hydride is widely applied to describe compounds of hydrogen with other elements, particularly those of groups 1–16. The variety of compounds formed by hydrogen is vast, arguably greater than that of any other element. Various metal hydrides are currently being studied for use as a means of hydrogen storage in fuel cell-powered electric cars and batteries. They also have important uses in organic chemistry as powerful reducing agents, and many promising uses in hydrogen economy.
Every element of the periodic table (except some noble gases) forms one or more hydrides. These compounds may be classified into three main types by the predominant nature of their bonding:
* Saline hydrides, which have significant ionic character,
* Covalent hydrides, which include the hydrocarbons and many other compounds, and
* Interstitial hydrides, which may be described as having metallic bonding.

HYDROCARBON: Any compound made of only carbon and hydrogen.

HYDROCHLORIC AND HYPOCHLOROUS ACIDS: The compounds that are formed in water when chlorine gas is introduced.

HYDROFLUOSILIC ACID: (H_2SiF_6) a clear, fuming corrosive liquid with a pH ranging from 1 to 1.5. Used in water treatment to fluoridate drinking water.

HYDROGEN BOND: A type of bond formed when the partially positive hydrogen atom of a polar covalent bond in one molecule is attracted to the partially negative atom of a polar covalent bond in another.

HYDROGEN SULFIDE OR CHLORINE GAS: These chemicals can cause olfactory fatigue.

HYDROGEN ION: A single proton with a charge of +1. The dissociation of a water molecule (H2O) leads to the generation of a hydroxide ion (OH-) and a hydrogen ion (H+).

HYDROGEN SULFIDE: A toxic gas formed by the anaerobic decomposition of organic matter. Chemical formula is H2S.

HYDROLYSIS: The chemical reaction that breaks a covalent bond through the addition of hydrogen (from a water molecule) to the atom forming one side of the original bond, and a hydroxyl group to the atom on the other side.

HYDROPHILIC: Having an affinity for water.

HYDROPHOBIC INTERACTION: A type of weak chemical bond formed when molecules that do not mix with water coalesce to exclude the water.

HYDROPHOBIC: The physicochemical property whereby a substance or region of a molecule resists association with water molecules.

HYDROSTATIC: Pertaining to the pressure and equilibrium of fluids. A hydrostatic skeleton is a skeletal system composed of fluid held under pressure in a closed body compartment; the main skeleton of most cnidarians, flatworms, nematodes, and annelids.

HYDROXYL GROUP: A functional group consisting of a hydrogen atom joined to an oxygen atom by a polar covalent bond. Molecules possessing this group are soluble in water and are called alcohols.

HYDROXYL ION: The OH- ion.

HYPEROSMOTIC: A solution with a greater solute concentration than another, a hypoosmotic solution. If the two solutions are separated from one another by a membrane permeable to water, water would tend to move from the hypo- to the hyperosmotic side.

HYPERPOLARIZATION: An electrical state whereby the inside of the cell is made more negative relative to the outside than was the case at resting potential. A neuron membrane is hyperpolarized if the voltage is increased from the resting potential of about -70 mV, reducing the chance that a nerve impulse will be transmitted.

HYPERTROPHY: Abnormal enlargement, excessive growth.

HYPHA: A fungal filament.

HYPOCHLORITE AND ORGANIC MATERIALS: Heat and possibly fire may occur when hypochlorite is brought into contact with an organic material.

HYPOCOTYL: The portion of the axis of a plant embryo below the point of attachment of the cotyledons; forms the base of the shoot and the root.

HYPOOSMOTIC SOLUTION: A solution with a lesser solute concentration than another, a hyperosmotic solution. If the two solutions are separated from one another by a membrane permeable to water, water would tend to move from the hypo- to the hyperosmotic side.

HYPOTHESIS: A formal statement of supposition offered to explain observations. Note that a hypothesis is only useful if it can be tested. Even if correct, it is not scientifically useful if untestable.

HYPOTHETICO-DEDUCTIVE: A method used to test hypotheses. If deductions formulated from the hypothesis are tested and proven false, the hypothesis is rejected.

IMAGINAL DISK: An island of undifferentiated cells in an insect larva, which are committed (determined) to form a particular organ during metamorphosis to the adult.

IMBIBITION: The soaking of water into a porous material that is hydrophilic.

IMMUNE RESPONSE: 1) A primary immune response is the initial response to an antigen, which appears after a lag of a few days. 2) A secondary immune response is the response elicited when the animal encounters the same antigen at a later time. The secondary response is normally more rapid, of greater magnitude and of longer duration than the primary response.

IMMUNOGLOBULINE: The class of proteins comprising the antibodies.

IMMUNOLOGICAL: 1) Immunological distance is the amount of difference between two proteins as measured by the strength of the antigen: antibody reaction between them. 2) Immunological tolerance is a mechanism by which an animal does not mount an immune response to the antigenic determinants of its own macromolecules.

IMMUNOMAGNETIC SEPARATION (IMS): A purification procedure that uses microscopic, magnetically responsive particles coated with an antibodies targeted to react with a specific pathogen in a fluid stream. Pathogens are selectively removed from other debris using a magnetic field.

IMPELLERS: The semi-open or closed props or blades of a turbine pump that when rotated generate the pumping force.

IMPERVIOUS: Not allowing, or allowing only with great difficulty, the movement of water.

IMPRINTING: A type of learned behavior with a significant innate component, acquired during a limited critical period.

IN SERIES: Several components being connected one to the other without a bypass, requiring each component to work dependent on the one before it.

IN SITU: Treatment or disposal methods that do not require movement of contaminated material.

IN VITRO FERTILIZATION: Fertilization of ova in laboratory containers followed by artificial implantation of the early embryo in the mother's uterus.

INCINERATION: The process of reducing the volume of a material by burning and reducing to ash if possible.

INCLINED PLATE SEPARATOR: A series of parallel inclined plates that can be used to increase the efficiency of clarifiers and gravity thickeners.

INCOMPLETE DOMINANCE: A type of inheritance in which F1 hybrids have an appearance that is intermediate between the phenotypes of the parental varieties.

INDETERMINATE: 1) A type of cleavage exhibited during the embryonic development in deuterostomes, in which each cell produced by early cleavage divisions retains the capacity to develop into a complete embryo; 2) A type of growth exhibited by plants: they continue to grow as long as they live, because they always retain meristematic cells capable of undergoing mitosis.

INDIRECT REUSE: The beneficial use of reclaimed water into natural surface waters or groundwater.

INDUCED FIT: The change in shape of the active site of an enzyme so that it binds more snugly to the substrate, induced by entry of the substrate.

INDUCTION: 1) The ability of one group of embryonic cells to influence the development of another. 2) A method in logic which proceeds from the specific to general and develops a general statement which explains all of the observations. Commonly used to formulate scientific hypotheses.

INDUSTRIAL MELANISM: Melanism which has resulted from blackening of environmental surfaces (tree bark, etc.) by industrial pollution. This favors survival of melanic forms such as moths which rest on tree bark and are less likely to be seen by predators.

INDUSTRIAL WASTEWATER: Liquid wastes resulting from industrial processes.

INFECTIOUS: 1) An infectious disease is a disease caused by an infectious microbial or parasitic agent. 2) Infectious hepatitis is the former name for hepatitis A. 3) Infectious mononucleosis is an acute disease that affects many systems, caused by the Epstein: Barr virus.

INFECTIOUS PATHOGENS/MICROBES/GERMS: are considered disease-producing bacteria, viruses and other microorganisms.

INFLAMMATORY RESPONSE: A line of defense triggered by penetration of the skin or mucous membranes, in which small blood vessels in the vicinity of an injury dilate and become leakier, enhancing infiltration of leukocytes; may also be widespread in the body.

INFLUENT: Water or wastewater flowing into a basin or treatment plant.

INGESTION: A heterotrophic mode of nutrition in which other organisms or detritus are eaten whole or in pieces.

INHIBITORY POSTSYNAPTIC POTENTIAL: An electrical charge (hyperpolarization) in the membrane of a postsynaptic neuron caused by the binding of an inhibitory neurotransmitter from a presynaptic cell to a postsynaptic receptor.

INITIAL PRECISION AND RECOVERY (IPR): Four aliquots of spiking suspension analyzed to establish the ability to generate acceptable precision and accuracy. An IPR is performed prior to the first time this method is used and any time the method or instrumentation is modified.

INNER CELL MASS: A cluster of cells in a mammalian blastocyst that protrudes into one end of the cavity and subsequently develops into the embryo proper and some of the extraembryonic membranes.

INORGANIC COMPOUND: Compounds that contain no carbon or contain only carbon bound to elements other than hydrogen.

INORGANIC CONTAMINANTS: Mineral-based compounds such as metals, nitrates, and asbestos. These contaminants are naturally-occurring in some water, but can also get into water through farming, chemical manufacturing, and other human activities. EPA has set legal limits on 15 inorganic contaminants.

INORGANIC IONS: Present in all waters. Inorganic ions are essential for human health in small quantities, but in larger quantities they can cause unpleasant taste and odor or even illness. Most community water systems will commonly test for the concentrations of seven inorganic ions: nitrate, nitrite, fluoride, phosphate, sulfate, chloride, and bromide. Nitrate and nitrite can cause an illness in infants called methemoglobinemia. Fluoride is actually added to the drinking water in some public water systems to promote dental health. Phosphate, sulfate, chloride, and bromide have little direct effect on health, but high concentrations of inorganic ions can give water a salty or briny taste.

INSOLUBLE COMPOUNDS: are types of compounds cannot be dissolved. When iron or manganese reacts with dissolved oxygen (**DO**) insoluble compound are formed.

INTAKE FACILITIES: One of the more important considerations in the construction of intake facilities is the ease of operation and maintenance over the expected lifetime of the facility. Every intake structure must be constructed with consideration for operator
safety and for cathodic protection.

INOSITOL TRIPHOSPHATE: The second messenger, which functions as an intermediate between certain non-steroid hormones and the third messenger, a rise in cytoplasmic Ca++ concentration.

INSERTION: A mutation involving the addition of one or more nucleotide pairs to a gene.

INSIGHT LEARNING: The ability of an animal to perform a correct or appropriate behavior on the first attempt in a situation with which it has had no prior experience.

INSULIN: The vertebrate hormone that lowers blood sugar levels by promoting the uptake of glucose by most body cells and promoting the synthesis and storage of glycogen in the liver; also stimulates protein and fat synthesis; secreted by endocrine cells of the pancreas called islets of Langerhans.

INTEGRAL PROTEIN: A protein of biological membranes that penetrates into or spans the membrane.

INTERBREED: To breed with another kind or species; hybridize.

INTERFERON: A chemical messenger of the immune system, produced by virus: infected cells and capable of helping other cells resist the virus.

INTERLEUKIN: 1: A chemical regulator (cytokine) secreted by macrophages that have ingested a pathogen or foreign molecule and have bound with a helper T cell; stimulates T cells to grow and divide and elevates body temperature. Interleukin: 2, secreted by activated T cells, stimulates helper T cells to proliferate more rapidly.

INTERMEDIATE FILAMENT: A component of the cytoskeleton that includes all filaments intermediate in size between microtubules and microfilaments.

INTERNEURON: An association neuron; a nerve cell within the central nervous system that forms synapses with sensory and motor neurons and integrates sensory input and motor output.

INTERNODE: The segment of a plant stem between the points where leaves are attached.

INTERSTITIAL CELLS: Cells scattered among the seminiferous tubules of the vertebrate testis that secrete testosterone and other androgens, the male sex hormones.

INTERSTITIAL FLUID: The internal environment of vertebrates consisting of the fluid filling the spaces between cells.

INTERTIDAL ZONE: The shallow zone of the ocean where land meets water.

INTRINSIC RATE OF INCREASE: The difference between number of births and number of deaths, symbolized as rmax; maximum population growth rate.

INTROGRESSION: Transplantation of genes between species resulting from fertile hybrids mating successfully with one of the parent species.

INTRON: The noncoding, intervening sequence of coding region (exon) in eukaryotic genes.

INVAGINATION: The buckling inward of a cell layer, caused by rearrangements of microfilaments and microtubules; an important phenomenon in embryonic development.

INVERSION: 1) An aberration in chromosome structure resulting from an error in meiosis or from mutagens; reattachment in a reverse orientation of a chromosomal fragment to the chromosome from which the fragment originated. 2) A phenomenon which occurs during early development of sponges at which time the external ciliated cells become inward-directed.

INVERTEBRATE: An animal without a backbone; invertebrates make up about 95% of animal species.

ION: A charged chemical formed when an atom or group of atoms has more or less electrons than protons (rather than an equal number).

ION EXCHANGE: An effective treatment process used to remove iron and manganese in a water supply. The hardness of the source water affects the amount of water an ion exchange softener may treat before the bed requires regeneration.

IONIC BOND: A chemical bond due to attraction between oppositely charged ions.

IRON: The elements iron and manganese are undesirable in water because they cause stains and promote the growth of iron bacteria.

IRON AND MANGANESE: In water they can usually be detected by observing the color of the inside walls of filters and the filter media. If the raw water is pre-chlorinated, there will be black stains on the walls below the water level and a black coating over the top portion of the sand filter bed. When significant levels of dissolved oxygen are present, iron and manganese exist in an oxidized state and normally precipitate into the reservoir bottom sediments. The presence of iron and manganese in water promote the growth of Iron bacteria. Only when a water sample has been acidified then you can perform the analysis beyond the 48 hour holding time. Iron and Manganese in water may be detected by observing the color of the of the filter media. Maintaining a free chlorine residual and regular flushing of water mains may control the growth of iron bacteria in a water distribution system.

IRRUPTION: A rapid increase in population density often followed by a mass emigration.

ISOGAMY: A condition in which male and female gametes are morphologically indistinguishable.

ISOMER: Molecules consisting of the same numbers and kinds of atoms, but differing in the way in which the atoms are combined.

ISOSMOTIC: Solutions of equal concentration with respect to osmotic pressure.

ISOTOPE: An atomic form of an element, containing a different number of neutrons than another isotope. Isotopes vary from one another with respect to atomic mass.

JUXTAGLOMERULAR APPARATUS (JGA): Specialized tissue located near the afferent arteriole that supplies blood to the kidney glomerulus; JGA raises blood pressure by producing renin, which activates angiotensin.

K- SELECTION: The concept that life history of the population is centered upon producing relatively few offspring that have a good chance of survival.

KARYOGAMY: The fusion of nuclei of two cells, as part of syngamy.

KARYOTYPE: A method of classifying the chromosomes of a cell in relation to number, size and type.

KEYSTONE PREDATOR: A species that maintains species richness in a community through predation of the best competitors in the community, thereby maintaining populations of less competitive species.

KILOCALORIE: A thousand calories; the amount of heat energy required to raise the temperature of 1 kilogram of water by primary C.

KILL = C X T: Where other factors are constant, the disinfecting action may be represented by: Kill=C x T.

KIN SELECTION: A phenomenon of inclusive fitness, used to explain altruistic behavior between related individuals.

KINESIS: A change in activity rate in response to a stimulus.

KINETIC ENERGY: The ability of an object to do work by virtue of its motion. The energy terms that are used to describe the operation of a pump are pressure and head. The energy of motion. Moving matter does work by transferring some of its kinetic energy to other matter.

KINETOCHORE: A specialized region on the centromere that links each sister chromatid to the mitotic spindle.

KINGDOM: A taxonomic category, the second broadest after domain.

KREBS CYCLE: A chemical cycle involving eight steps that completes the metabolic breakdown of glucose molecules to carbon dioxide; occurs within the mitochondrion; the second major stage in cellular respiration. Also called citric acid cycle or tricarboxylic acid (TCA) cycle.

LABORATORY BLANK: See Method blank

LABORATORY CONTROL SAMPLE (LCS): See Ongoing precision and recovery (OPR) standard

LACTEAL: A tiny lymph vessel extending into the core of the intestinal villus and serving as the destination for absorbed chylomicrons.

LACTIC ACID: Gram-positive, anaerobic; produce lactic acid through fermentation; include Lactobacillus, essential in dairy product formation, and Streptococcus, common in humans.

LAGGING STRAND: A discontinuously synthesized DNA strand that elongates in a direction away from the replication fork.

LAMARCK: Proposed, in the early 1800s, that evolutionary change may occur via the inheritance of acquired characteristics. This idea, which has since been discredited, holds that the changes in characteristics which occur during an individual's life can be passed on to its offspring.

LAND APPLICATION: The disposal of wastewater or municipal solids onto land under controlled conditions.

LAND DISPOSAL: Application of municipal wastewater solids to the soil without production of usable agricultural products.

LANDFILL: A land disposal site that employs an engineering method of solid waste disposal to minimize environmental hazards and protect the quality of surface and subsurface waters.

LANGELIER INDEX: A measurement of Corrosivity. The water is becoming corrosive in the distribution system causing rusty water if the Langelier index indicates that the pH has decreased from the equilibrium point. Mathematically derived factor obtained from the values of calcium hardness, total alkalinity, and pH at a given temperature. A Langelier index of zero indicates perfect water balance (i.e., neither corroding nor scaling). The Langelier Saturation Index (sometimes Langelier Stability Index) is a calculated number used to predict the calcium carbonate stability of water. It indicates whether the water will precipitate, dissolve, or be in equilibrium with calcium carbonate. Langelier developed a method for predicting the pH at which water is saturated in calcium carbonate (called pHs). The LSI is expressed as the difference between the actual system pH and the saturation pH.
LSI = pH - pHs
If the actual pH of the water is below the calculated saturation pH, the LSI is negative and the water has a very limited scaling potential. If the actual pH exceeds pHs, the LSI is positive, and being supersaturated with CaCO3, the water has a tendency to form scale. At increasing positive index values, the scaling potential increases.
Langelier saturation index is defined as:
LSI = pH (measured) - pHs
* For LSI > 0, water is super saturated and tends to precipitate a scale layer of $CaCO_3$
* For LSI = 0, water is saturated (in equilibrium) with $CaCO_3$. A scale layer of $CaCO_3$ is neither precipitated nor dissolved
* For LSI < 0, water is under saturated and tends to dissolve solid $CaCO_3$
In practice, water with an LSI between -0.5 and +0.5 will not display enhanced mineral dissolving or scale forming properties. Water with an LSI below -0.5 tends to exhibit noticeably increased dissolving abilities while water with an LSI above +0.5 tends to exhibit noticeably increased scale forming properties.

It is also worth noting that the LSI is temperature sensitive. The LSI becomes more positive as the water temperature increases. This has particular implications in situations where well water is used. The temperature of the water when it first exits the well is often significantly lower than the temperature inside the building served by the well or at the laboratory where the LSI measurement is made.

LARVA (pl. larvae): A free-living, sexually immature form in some animal life cycles that may differ from the adult in morphology, nutrition, and habitat.

LATERAL LINE SYSTEM: A mechanoreceptor system consisting of a series of pores and receptor units (neuromasts) along the sides of the body of fishes and aquatic amphibians; detects water movements made by an animal itself and by other moving objects.

LATERAL MERISTEMS: The vascular and cork cambia, cylinders of dividing cells that run most of the length of stems and roots and are responsible for secondary growth.

LAW OF INDEPENDENT ASSORTMENT: Mendel's second law, stating that each allele pair segregates independently during gamete formation; applies when genes for two traits are located on different pairs of homologous chromosomes.

LAW OF SEGREGATION: Mendel's first law, stating that allele pairs separate during gamete formation, and then randomly re-form pairs during the fusion of gametes at fertilization.

LEACHATE: Fluid that trickles through solid materials or wastes and contains suspended or dissolved materials or products of the solids.

LEACHING: A chemical reaction between water and metals that allows for removal of soluble materials.

LEADING STRAND: The new continuously complementary DNA strand synthesized along the template strand in the 5' ---- > 3' direction.

LEUKOCYTE: A white blood cell; typically functions in immunity, such as phagocytosis or antibody production.

LEVELS OF ORGANIZATION: A basic concept in biology is that organization is based on a hierarchy of structural levels, with each level building on the levels below it.

LICHEN: An organism formed by the symbiotic association between a fungus and a photosynthetic alga.

LIFE: A table of data summarizing mortality in a population.

LIGAMENT: A type of fibrous connective tissue that joins bones together at joints.

LIGAND: A ligand is a molecule that binds specifically to a receptor site of another molecule. A ligase is an enzyme which catalyzes such a reaction. For example, a DNA ligase is an enzyme which catalyzes the covalent bonding of the 3' end of a new DNA fragment to the 5' end of a growing chain.

LIGASE: Ligases are enzymes that catalyze the "stitching together" of polymer fragments. DNA ligase, for example, catalyzes phosphodiester bond formation between two DNA fragments, and this enzyme is involved in normal DNA replication, repair of damaged chromosomes, and various in vitro techniques in genetic engineering that involve linking DNA fragments.

LIGNIN: A hard material embedded in the cellulose matrix of vascular plant cell walls that functions as an important adaptation for support in terrestrial species.

LIMBIC SYSTEM: A group of nuclei (clusters of nerve cell bodies) in the lower part of the mammalian forebrain that interact with the cerebral cortex in determining emotions; includes the hippocampus and the amygdala.

LIME: The term generally used to describe ground limestone (calcium carbonate), hydrated lime (calcium hydroxide), or burned lime (calcium oxide).

LIME SOFTENING: Lime softening is primarily used to “soften” water—that is to remove calcium and magnesium mineral salts. But it also removes harmful toxins like radon and arsenic. Though there is no consensus, some studies have even suggested that lime softening is effective at removal of Giardia. Hard water is a common condition responsible for numerous problems. Users often recognize hard water because it prevents their soap from lathering properly. However, it can also cause buildup (“scale”) in hot water heaters, boilers, and hot water pipes. Because of these inconveniences, many treatment facilities use lime softening to soften hard water for consumer use. Before lime softening can be used, managers must determine the softening chemistry required. This is a relatively easy task for groundwater sources, which remain more constant in their composition. Surface waters, however, fluctuate widely in quality and may require frequent changes to the softening chemical mix. In lime softening, lime and sometimes sodium carbonate are added to the water as it enters a combination solids contact clarifier. This raises the pH (i.e., increases alkalinity) and leads to the precipitation of calcium carbonate. Later, the pH of the effluent from the clarifier is reduced again, and the water is then filtered through a granular media filter. The water chemistry requirements of these systems require knowledgeable operators, which may make lime softening an economic challenge for some very small systems.

LIME STABILIZATION: The addition of lime to untreated sludge to raise the pH to 12 for a minimum of 2 hours to chemically inactivate microorganisms.

LINKED GENES: Genes that are located on the same chromosomes.

LIPID: One of a family of compounds, including fats, phospholipids, and steroids, that are insoluble in water.

LIPOPROTEIN: A protein bonded to a lipid; includes the low-density lipoproteins (LDLS) and high-density lipoproteins (HDLS) that transport fats and cholesterol in the blood.

LIPOSOME: Liposomes are vesicles (spherules) in which the lipid molecules are spontaneously arranged into bilayers with hydrophilic groups exposed to water molecules both outside the vesicle and in the core.

LISTED HAZARDOUS WASTE: The designation for a waste material that appears on an EPA list of specific hazardous wastes or hazardous waste categories.

LOCUS: A particular place along the length of a certain chromosome where a specified allele is located.

LOGISTIC POPULATION GROWTH: A model describing population growth that levels off as population size approaches carrying capacity.

L.O.T.O.: If a piece of equipment is locked out, the key to the lock-out device the key should be held by the person who is working on the equipment. The tag is an identification device and the lock is a physical restraint.

LYMPHOCYTE: Lymphocytes (lymph cells, lympho- leukocytes) are a type of leukocyte (white blood cell) responsible for the immune response. There are two classes of lymphocytes: 1) the B- cells, when presented with a foreign chemical entity (antigen), change into antibody producing plasma cells; and, 2) the T- cells interact directly with foreign invaders such as bacteria and viruses. The T- cells express various surface marker macromolecules. For example, CD4+ is the notation for a specific expressed T- cell surface marker that can be identified by assay.

LYSIS: The destruction of a cell by rupture of the plasma membrane.

LYSOGENIC CYCLE: A type of viral replication cycle in which the viral genome becomes incorporated into the bacterial host chromosome as a prophage.

LYSOSOME: A membrane-bounded organelle found in eukaryotic cells (other than plants). Lysosomes contain a mixture of enzymes that can digest most of the macromolecules found in the rest of the cell.

LYSOZYME: An enzyme in perspiration, tears, and saliva that attacks bacterial cell walls.

LYTIC CYCLE: A type of viral replication cycle resulting in the release of new phages by death or lysis of the host cell.

M-ENDO BROTH: The coliform group are used as indicators of fecal pollution in water, for assessing the effectiveness of water treatment and disinfection, and for monitoring water quality. m-Endo Broth is used for selectively isolating coliform bacteria from water and other specimens using the membrane filtration technique. m-Endo Broth is prepared according to the formula of Fifield and Schaufus.1 It is recommended by the American Public Health Association in standard total coliform membrane filtration procedure for testing water, wastewater, and foods.2,3 The US EPA specifies using m-Endo Broth in the total coliform methods for testing water using single-step, two-step, and delayed incubation membrane filtration methods.

M PHASE: The mitotic phase of the cell cycle, which includes mitosis and cytokinesis.

MACROEVOLUTION: Evolutionary change on a grand scale, encompassing the origin of novel designs, evolutionary trends, adaptive radiation, and mass extinction.

MACROMOLECULE: A giant molecule of living matter formed by the joining of smaller molecules, usually by condensation synthesis. Polysaccharides, proteins, and nucleic acids are macromolecules.

MACROPHAGE: An amoeboid cell that moves through tissue fibers, engulfing bacteria and dead cells by phagocytosis.

MAGNESIUM HARDNESS: Measure of the magnesium salts dissolved in water – it is not a factor in water balance.

MAGNETIC STARTER: Is a type of motor starter should be used in an integrated circuit to control flow automatically.

MAJOR HISTOCOMPATIBILITY COMPLEX: A large set of cell surface antigens encoded by a family of genes. Foreign MHC markers trigger T-cell responses that may lead to rejection of transplanted tissues and organs.

MAKEUP WATER: Fluid introduced in a recirculating stream to maintain an equilibrium of temperature, solids concentration or other parameters. Also refers to the quantity of water required to make a solution.

MALIGNANT TUMOR: A cancerous growth; an abnormal growth whose cells multiply excessively, have altered surfaces, and may have unusual numbers of chromosomes and/or aberrant metabolic processes.

MALPHIGHIAN TUBULE: A unique excretory organ of insects that empties into the digestive tract, removes nitrogenous wastes from the blood, and functions in osmoregulation.

MANTLE: A heavy fold of tissue in mollusks that drapes over the visceral mass and may secrete a shell.

MARBLE AND LANGELIER TESTS: Are used to measure or determine the corrosiveness of a water source.

MASS NUMBER: The sum of the number of protons plus the number of neutrons in the nucleus of an atom; unique for each element and designated by a superscript to the left of the elemental symbol.

MATRIX SPIKE (MS): A sample prepared by adding a known quantity of organisms to a specified amount of sample matrix for which an independent estimate of target analyte concentration is available. A matrix spike is used to determine the effect of the matrix on a method's recovery efficiency.

MATRIX: The nonliving component of connective tissue, consisting of a web of fibers embedded in homogeneous ground substance that may be liquid, jellylike, or solid.

MATTER: Anything that takes up space and has mass.

MAXIMUM CONTAMINANT LEVEL (MCLs): The maximum allowable level of a contaminant

MECHANICAL SEAL: A mechanical device used to control leakage from the stuffing box of a pump. Usually made of two flat surfaces, one of which rotates on the shaft. The two flat surfaces are of such tolerances as to prevent the passage of water between them. Held in place with spring pressure.

MECHANORECEPTOR: A sensory receptor that detects physical deformations in the body environment associated with pressure, touch, stretch, motion, and sound.

MEDIAN BODIES: Prominent, dark-staining, paired organelles consisting of microtubules and found in the posterior half of *Giardia*. In *G. intestinalis* (from humans), these structures often have a claw-hammer shape, while in *G. muris* (from mice), the median bodies are round.

MEDIUM WATER SYSTEM: More than 3,300 persons and 50,000 or fewer persons.

MEDULLA OBLONGATA: The lowest part of the vertebrate brain; a swelling of the hindbrain dorsal to the anterior spinal cord that controls autonomic, homeostatic functions, including breathing, heart and blood vessel activity, swallowing, digestion, and vomiting.

MEDUSA: The floating, flattened, mouth-down version of the cnidarian body plan. The alternate form is the polyp.

MEGAPASCAL: A unit of pressure equivalent to 10 atmospheres of pressure.

MEGGER: Used to test the insulation resistance on a motor.

MEIOSIS: A two-stage type of cell division in sexually reproducing organisms that results in gametes with half the chromosome number of the original cell.

MEMBRANE: A thin barrier that permits passage of particles of a certain size or of particular physical or chemical properties.

MEMBRANE POTENTIAL: The charge difference between the cytoplasm and extracellular fluid in all cells, due to the differential distribution of ions. Membrane potential affects the activity of excitable cells and the transmembrane movement of all charged substances.

MESENTERIES: Membranes that suspend many of the organs of vertebrates inside fluid- filled body cavities.

MESODERM: The middle primary germ layer of an early embryo that develops into the notochord, the lining of the coelom, muscles, skeleton, gonads, kidneys and most of the circulatory system.

MESOSOME: A localized infolding of the plasma membrane of a bacterium.

MESSENGER: (RNA) A type of RNA synthesized from DNA in the genetic material that attaches to ribosomes in the cytoplasm and specifies the primary structure of a protein.

METABOLISM: The sum total of the chemical and physical changes constantly taking place in living substances.

METALLOID: Metalloid is a term used in chemistry when classifying the chemical elements. On the basis of their general physical and chemical properties, nearly every element in the periodic table can be termed either a metal or a nonmetal. A few elements with intermediate properties are, however, referred to as metalloids. (In Greek metallon = metal and eidos = sort)
There is no rigorous definition of the term, but the following properties are usually considered characteristic of metalloids:
* metalloids often form amphoteric oxides.
* metalloids often behave as semiconductors (B,Si,Ge) to semimetals (e.g. Sb).
The concepts of metalloid and semiconductor should not be confused. Metalloid refers to the properties of certain elements in relation to the periodic table. Semiconductor refers to the physical properties of materials (including alloys, compounds) and there is only partial overlap between the two.

METAMORPHOSIS: The resurgence of development in an animal larva that transforms it into a sexually mature adult.

METANEPHRIDIUM: A type of excretory tubule in annelid worms that has internal openings called nephrostomes that collect body fluids and external openings called nephridiopores.

METASTASIS: The spread of cancer cells beyond their original site.

METAZOAN: A multicellular animal. Among important distinguishing characteristics of metazoa are cell differentiation and intercellular communication. For certain multicellular colonial entities such as sponges, some biologists prefer the term "parazoa".

METHANE: Methane is a chemical compound with the molecular formula CH4. It is the simplest alkane, and the principal component of natural gas. Methane's bond angles are 109.5 degrees. Burning methane in the presence of oxygen produces carbon dioxide and water. The relative abundance of methane and its clean burning process makes it a very attractive fuel. However, because it is a gas at normal temperature and pressure, methane is difficult to transport from its source.

In its natural gas form, it is generally transported in bulk by pipeline or LNG carriers; few countries still transport it by truck. Methane is a relatively potent greenhouse gas with a high global warming potential of 72 (averaged over 20 years) or 25 (averaged over 100 years).[1] Methane in the atmosphere is eventually oxidized, producing carbon dioxide and water. As a result, methane in the atmosphere has a half-life of seven years (if no methane was added, then every seven years, the amount of methane would halve). The abundance of methane in the Earth's atmosphere in 1998 was 1745 parts per billion, up from 700 ppb in 1750. In the same time period, CO2 increased from 278 to 365 parts per million. The radiative forcing effect due to this increase in methane abundance is about one-third of that of the CO2 increase. In addition, there is a large, but unknown, amount of methane in methane clathrates in the ocean floors. Global warming could release this methane, which could cause a further sharp rise in global temperatures. Such releases of methane may have been a major factor in previous major extinction events. The Earth's crust also contains huge amounts of methane. Large amounts of methane are produced anaerobically by methanogenesis. Other sources include mud volcanoes which are connected with deep geological faults.

METHOD BLANK: An aliquot of reagent water that is treated exactly as a sample, including exposure to all glassware, equipment, solvents, and procedures that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Mg/L: Stands for "**milligrams per liter**." A common unit of chemical concentration. It expresses the mass of a chemical that is present in a given volume of water. A milligram (one one-thousandth of a gram) is equivalent to about 18 grains of table salt. A liter is equivalent to about one quart.

MICROBE OR MICROBIAL: Any minute, simple, single-celled form of life, especially one that causes disease.

MICROBIAL CONTAMINANTS: Microscopic organisms present in untreated water that can cause waterborne diseases.

MICROBIOLOGICAL: Is a type of analysis in which a composite sample unacceptable.

MICROBODY: A small organelle, bounded by a single membrane and possessing a granular interior. Peroxisomes and glyoxysomes are types of microbodies.

MICROEVOLUTION: A change in the gene pool of a population over a succession of generations.

MICROFILAMENT: Minute fibrous structure generally composed of actin found in the cytoplasm of eukaryotic cells. They play a role in motion within cells.

MICROFILTRATION: A low pressure membrane filtration process that removes suspended solids and colloids generally larger than 0.1 micron diameter.

MICROORGANISMS: Very small animals and plants that are too small to be seen by the naked eye and must be observed using a microscope. Microorganisms in water include algae, bacteria, viruses, and protozoa. Algae growing in surface waters can cause off-taste and odor by producing the chemicals MIB and geosmin. Certain types of bacteria, viruses, and protozoa can cause disease in humans. Bacteria are the most common microorganisms found in treated drinking water. The great majority of bacteria are not harmful. In fact, humans would not be able to live without the bacteria that inhabit the intestines. However, certain types of bacteria called coliform bacteria can signal the presence of possible drinking water contamination.

MICROSCOPE: An instrument which magnifies images either by using lenses in an optical system to bend light (light microscope) or electromagnets to direct the movement of electrons (electron microscope).

MICROTUBULE: A minute tubular structure found in centrioles, spindle apparati, cilia, flagella, and other places in the cytoplasm of eukaryotic cells. Microtubules play a role in movement and maintenance of shape.

MICROVILLUS: Collectively, fine, fingerlike projections of the epithelial cells in the lumen of the small intestine that increase its surface area.

MILLIGRAMS PER LITER: (mg/L) A common unit of measurement of the concentration of a material in solution.

MILLILITER: One one-thousandth of a liter; A liter is a little more than a quart. A milliliter is about two drops from an eye dropper.

MIMICRY: A phenomenon in which one species benefits by a superficial resemblance to an unrelated species. A predator or species of prey may gain a significant advantage through mimicry.

MISCIBLE: Capable of being mixed together.

MISSENSE: (mutation) The most common type of mutation involving a base- pair substitution within a gene that changes a codon, but the new codon makes sense, in that it still codes for an amino acid.

MITOCHONDRIAL MATRIX: The compartment of the mitochondrion enclosed by the inner membrane and containing enzymes and substrates for the Krebs cycle.

MITOCHONDRION: An organelle that occurs in eukaryotic cells and contains the enzymes of the citric acid cycle, the respiratory chain, and oxidative phosphorylation. A mitochondrion is bounded by a double membrane.

MITOSIS: A process of cell division in eukaryotic cells conventionally divided into the growth period (interphase) and four stages: prophase, metaphase, anaphase, and telophase. The stages conserve chromosome number by equally allocating replicated chromosomes to each of the daughter cells.

MIXED LIQUOR SUSPENDED SOLIDS: Suspended solids in the mixture of wastewater and activated sludge undergoing aeration in the aeration basin.

MODEM SYNTHESIS: A comprehensive theory of evolution emphasizing natural selection, gradualism, and populations as the fundamental units of evolutionary change; also called Neo-Darwinism.

MOISTURE: If a material is hygroscopic, it must it be protected from water.

MOISTURE AND POTASSIUM PERMANGANATE: The combination of moisture and potassium permanganate produces heat.

MOLARITY: A common measure of solute concentration, referring to the number of moles of solute in 1 L of solution.

MOLD: A rapidly growing, asexually reproducing fungus.

MOLE: The number of grams of a substance that equals its molecular weight in daltons and contains Avogadro's number of molecules.

MOLECULAR FORMULA: A type of molecular notation indicating only the quantity of the constituent atoms.

MOLECULAR WEIGHT: The molecular mass (abbreviated Mr) of a substance, formerly also called molecular weight and abbreviated as MW, is the mass of one molecule of that substance, relative to the unified atomic mass unit u (equal to 1/12 the mass of one atom of carbon-12). This is distinct from the relative molecular mass of a molecule, which is the ratio of the mass of that molecule to 1/12 of the mass of carbon 12 and is a dimensionless number. Relative molecular mass is abbreviated to Mr.

MOLECULE: Two or more atoms of one or more elements held together by ionic or covalent chemical bonds.

MOLTING: A process in arthropods in which the exoskeleton is shed at intervals to allow growth by secretion of a larger exoskeleton.

MONERA: The kingdom of life forms that includes all of the bacteria.

MONOCLONAL ANTIBODY: A defensive protein produced by cells descended from a single cell; an antibody that is secreted by a clone of cells and, consequently, is specific for a single antigenic determinant.

MONOECIOUS: Referring to an organism having the capacity of producing both sperm and eggs.

MONOHYBRID CROSS: A breeding experiment that employs parental varieties differing in a single character.

MONOMER: A small molecule, two or more of which can be combined to form oligomers (consisting of a few monomers) or polymers (consisting of many monomers).

MONOPHYLETIC: A term used to describe any taxon derived from a single ancestral form that gave rise to no species in other taxa.

MONOSACCHARIDE: A simple sugar; a monomer.

MONOZYGOTIC TWINS: Monozygotic twins are genetically identical, derived from the division and autonomous development of a single zygote (fertilized egg).

MORPHOGENESIS: The development of body shape and organization during ontogeny.

MORPHOSPECIES: Species defined by their anatomical features.

MOSAIC: A pattern of development, such as that of a mollusk, in which the early blastomeres each give rise to a specific part of the embryo. In some animals, the fate of the blastomeres is established in the zygote.

MOTOR NERVOUS SYSTEM: In vertebrates, the component of the peripheral nervous system that transmits signals from the central nervous system to effector cells.

MPF: M: phase promoting factor: A protein complex required for a cell to progress from late interphase to mitosis; the active form consists of cyclin and cdc2, a protein kinase.

M.S.D.S.: A safety document must an employer provide to an operator upon request.

MUCOSA: Refers to the mucous tissue lining various tubular structures in the body.

MUD BALLS IN FILTER MEDIA: Is a possible result of an ineffective or inadequate filter backwash.

MULLERIAN MIMICRY: A mutual mimicry by two unpalatable species.

MULTIGENE FAMILY: A collection of genes with similar or identical sequences, presumably of common origin.

MUNICIPAL WASTE: The combined solid and liquid waste from residential, commercial and industrial sources.

MUNICIPAL WASTEWATER TREATMENT PLANT (MWTP): Treatment works designed to treat municipal wastewater.

MURIATIC ACID: An acid used to reduce pH and alkalinity. Also used to remove stain and scale.

MUST: This action, activity, or procedural step is required.

MUTAGEN: A chemical or physical agent that interacts with DNA and causes a mutation.

MUTAGENESIS: The creation of mutations.

MUTATION: A spontaneous or induced change in a gene's or chromosome's structure or number. The resulting individual is termed a mutant.

MUTUALISM: A symbiotic relationship in which both the host and the symbiont benefit.

MYCELIUM: The densely branched network of hyphae in a fungus.

MYCOBACTERIUM: Pleomorphic spherical or rod-shaped, frequently branching, no gram stain, aerobic; commonly form yellow pigments; include Mycobacterium tuberculosis, cause of tuberculosis.

MYCOPLASMA: Spherical, commonly forming branching chains, no gram stain, aerobic but can live in certain anaerobic conditions; without cell walls yet structurally resistant to lysis; among smallest of bacteria; named for superficial resemblance to fungal hyphae (myco-means “fungus’).

MYELIN SHEATH: An insulating coat of cell membrane from Schwann cells that is interrupted by nodes of Ranvier where saltatory conduction occurs.

MYOFIBRILS: Fibrils arranged in longitudinal bundles in muscle cells (fibers); composed of thin filaments of actin and a regulatory protein and thick filaments of myosin.

MYOGLOBIN: An oxygen-storing, pigmented protein in muscle cells.

MYOSIN: A type of protein filament that interacts with actin filaments to cause cell movement, such as contraction in muscle cells.

NAD+: Nicatinamide adenine dinucleotide (oxidized); a coenzyme present in all cells that assists enzymes in transferring electrons during the redox reactions of metabolism.

NANO-FILTRATION: A specialty membrane filtration process that rejects solutes larger than approximately one nanometer (10 angstroms) in size.

NANOMETER: A unit of measure (length). 1 nm is equal to 1 x 10: 9 m, or 1/1,000,000 mm.

NaOCl: Is the molecular formula of Sodium hypochlorite.

NaOH: Is the molecular formula of Sodium hydroxide.

NATURAL ORGANIC MATTER: Organic matter present in natural waters.

NEGATIVE CONTROL: See Method blank.

NEGATIVE FEEDBACK: A primary mechanism of homeostasis, whereby a change in a physiological variable that is being monitored triggers a response that counteracts the initial fluctuation.

NEURAMINIDASE: A surface enzyme possessed by some influenza viruses which help the virus penetrate the mucus layer protecting the respiratory epithelium and also plays a role in budding of new virus particles from infected cells.

NEUTRALIZATION: The chemical process that produces a solution that is neither acidic nor alkaline. Usually with a pH between 6 and 8.

NEURON: A nerve cell; the fundamental unit of the nervous system, having structure and properties that allow it to conduct signals by taking advantage of the electrical charge across its cell membrane.

NEUROSECRETORY CELLS: Cells that receive signals from other nerve cells, but instead of signaling to an adjacent nerve cell or muscle, release hormones into the blood stream.

NEUROTRANSMITTER: The chemical messenger released from the synaptic terminals of a neuron at a chemical synapse that diffuses across the synaptic cleft and binds to and stimulates the postsynaptic cell.

NEUTRAL VARIATION: Genetic diversity that confers no apparent selective advantage.

NEUTRALIZATION REACTIONS: Chemical reactions between acids and bases where water is an end product.

NEUTRON: An uncharged subatomic particle of about the same size and mass as a proton.

NH3: The molecular formula of Ammonia.

NH4+: The molecular formula of the Ammonium ion.

NITRATES: A dissolved form of nitrogen found in fertilizers and sewage by-products that may leach into groundwater and other water sources. Nitrates may also occur naturally in some waters. Over time, nitrates can accumulate in aquifers and contaminate groundwater.

NITROGEN: Nitrogen is a nonmetal, with an electronegativity of 3.0. It has five electrons in its outer shell and is therefore trivalent in most compounds. The triple bond in molecular nitrogen (N2) is one of the strongest in nature. The resulting difficulty of converting (N_2) into other compounds, and the ease (and associated high energy release) of converting nitrogen compounds into elemental N2, have dominated the role of nitrogen in both nature and human economic activities. At atmospheric pressure molecular nitrogen condenses (liquefies) at 77 K (-195.8 °C) and freezes at 63 K (-210.0 °C) into the beta hexagonal close-packed crystal allotropic form. Below 35.4 K (-237.6 °C) nitrogen assumes the alpha cubic crystal allotropic form. Liquid nitrogen, a fluid resembling water, but with 80.8% of the density, is a common cryogen. Unstable allotropes of nitrogen consisting of more than two nitrogen atoms have been produced in the laboratory, like N3 and N4.[1] Under extremely high pressures (1.1 million atm) and high temperatures (2000 K), as produced under diamond anvil conditions, nitrogen polymerizes into the single bonded diamond crystal structure, an allotrope nicknamed "nitrogen diamond."

NITROGEN AND PHOSPHORUS: Pairs of elements and major plant nutrients that cause algae to grow.

NITROGEN-FIXING: Rod-shaped, gram-negative, aerobic; convert atmospheric nitrogen gas to ammonium in soil; include Azotobacter, a common genus.

NO3-: The molecular formula of the Nitrate ion.

NOMENCLATURE: The method of assigning names in the classification of organisms.

NON-CARBONATE HARDNESS: The portion of the total hardness in excess of the alkalinity.

NON-CARBONATE IONS: Water contains non-carbonate ions if it cannot be softened to a desired level through the use of lime only.

NON-POINT SOURCE POLLUTION: Air pollution may leave contaminants on highway surfaces. This non-point source pollution adversely impacts reservoir water and groundwater quality.

NONCOMPETITIVE INHIBITOR: A substance that reduces the activity of an enzyme by binding to a location remote from the active site, changing its conformation so that it no longer binds to the substrate.

NONCYCLIC ELECTRON FLOW: A route of electron flow during the light reactions of photosynthesis that involves both photosystems and produces ATP, NADPH, and oxygen; the net electron flow is from water to NADP+.

NONCYCLIC PHOTOPHOSPHORYLATION: The production of ATP by noncyclic electron flow.

NONDISJUNCTION: An accident of meiosis or mitosis, in which both members of a pair of homologous chromosomes or both sister chromatids fail to separate normally.

NONPOLAR: Electrically symmetrical. For example, in many molecules with covalent bonds, the electrons are shared equally; the poles are electrically neutral.

NONSENSE MUTATION: A mutation that changes an amino acid codon to one of the three stop codons, resulting in a shorter and usually nonfunctional protein.

NORM OF REACTION: The range of phenotypic possibilities for a single genotpe, as influenced by the environment.

NORMALITY: It is the number of equivalent weights of solute per liter of solution. Normality highlights the chemical nature of salts: in solution, salts dissociate into distinct reactive species (ions such as H+, Fe3+, or Cl-). Normality accounts for any discrepancy between the concentrations of the various ionic species in a solution. For example, in a salt such as MgCl2, there are two moles of Cl- for every mole of Mg2+, so the concentration of Cl- as well as of Mg2+ is said to be 2 N (read: "two normal"). Further examples are given below. A normal is one gram equivalent of a solute per liter of solution. The definition of a gram equivalent varies depending on the type of chemical reaction that is discussed - it can refer to acids, bases, redox species, and ions that will precipitate. It is critical to note that normality measures a single ion which takes part in an overall solute. For example, one could determine the normality of hydroxide or sodium in an aqueous solution of sodium hydroxide, but the normality of sodium hydroxide itself has no meaning. Nevertheless it is often used to describe solutions of acids or bases, in those cases it is implied that the normality refers to the H+ or OH- ion. For example, 2 Normal sulfuric acid (H_2SO_4), means that the normality of H+ ions is 2, or that the molarity of the sulfuric acid is 1. Similarly for 1 Molar H_3PO_4 the normality is 3 as it contains three H+ ions.

NTU: (Nephelometric turbidity unit)**:** A measure of the clarity or cloudiness of water.

NUCLEAR: 1) (envelope) The surface, consisting of two layers of membrane, that encloses the nucleus of eukaryotic cells. 2) (pore) An opening of the nuclear envelope which allows for the movement of materials between the nucleus and surrounding cytoplasm.

NUCLEASE: This term refers to any enzyme that acts on nucleic acids, e.g., Dnase, Rnase, endonuclease, etc.

NUCLEIC: (acid) A polymer composed of nucleotides that are joined by covalent bonds (phosphodiester linkages) between the phosphate of one nucleotide and the sugar of the next nucleotide.

NUCLEOID: The region that harbors the chromosome of a prokaryotic cell. Unlike the eukaryotic nucleus, it is not bounded by a membrane.

NUCLEOLUS (pl. nucleoli): A specialized structure in the nucleus, formed from various chromosomes and active in the synthesis of ribosomes.

NUCLELUS: A small, generally spherical body found within the nucleus of eukaryotic cells. The site of ribosomal RNA synthesis.

NUCLEOSIDE: An organic molecule consisting of a nitrogenous base joined to a five- carbon sugar.

NUCLEOSOME: The basic, beadlike unit of DNA packaging in eukaryotes, consisting of a segment of DNA wound around a protein core composed of two copies of each of four types of histone.

NUCLEOTIDE: The basic chemical unit (monomer) of a nucleic acid. A nucleotide in RNA consists of one of four nitrogenous bases linked to ribose, which in turn is linked to phosphate. In DNA, deoxyribose is present instead of ribose.

NUCLEUS: A membrane-bound organelle containing genetic material. Nuclei are a prominent internal structure seen both in *Cryptosporidium* oocysts and *Giardia* cysts. In *Cryptosporidium* oocysts, there is one nucleus per sporozoite. One to four nuclei can be seen in *Giardia* cysts.

NUCLEUS: The membrane bound organelle of eukaryotic cells that contains the cell's genetic material. Also the central region of an atom composed of protons and neutrons.

NULL: In the scientific method, the hypothesis which one attempts to falsify.

O_3: The molecular formula of ozone.

OLIGOTROPHIC: A reservoir that is nutrient-poor and contains little plant or animal life. An oligotrophic ecosystem or environment is one that offers little to sustain life. The term is commonly utilized to describe bodies of water or soils with very low nutrient levels. It derives etymologically from the Greek oligo (small, little, few) and trophe (nutrients, food). Oligotrophic environments are of special interest for the alternative energy sources and survival strategies upon which life could rely.

ONGOING PRECISION AND RECOVERY (OPR) STANDARD: A method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

OOCYST AND CYST SPIKING SUSPENSION: See Spiking suspension.

OOCYST AND CYST STOCK SUSPENSION: See Stock suspension.

OOCYST: The encysted zygote of some sporozoa; e.g., *Cryptosporidium*. The oocyst is a phase or form of the organism produced as a normal part of the life cycle of the organism. It is characterized by a thick and environmentally resistant outer wall.

ORGANIC: Relating to, or derived from, a living thing. A description of a substance that contains carbon atoms linked together by carbon-carbon bonds.

ORGANIC MATTER: Substances containing carbon compounds, usually of animal or vegetable origin.

ORGANIC PRESURSORS: Natural or man-made compounds with chemical structures based upon carbon that, upon combination with chlorine, leading to trihalomethane formation.

OSMOSIS: Osmosis is the process by which water moves across a semi permeable membrane from a low concentration solute to a high concentration solute to satisfy the pressure differences caused by the solute.

OVER-RANGE PROTECTION DEVICES: Mechanical dampers, snubbers and an air cushion chamber are examples of surging and overrange protection devices.

OXIDE: An oxide is a chemical compound containing at least one oxygen atom as well as at least one other element. Most of the Earth's crust consists of oxides. Oxides result when elements are oxidized by oxygen in air. Combustion of hydrocarbons affords the two principal oxides of carbon, carbon monoxide and carbon dioxide. Even materials that are considered to be pure elements often contain a coating of oxides. For example, aluminum foil has a thin skin of Al2O3 that protects the foil from further corrosion. Virtually all elements burn in an atmosphere of oxygen. In the presence of water and oxygen (or simply air), some elements - lithium, sodium, potassium, rubidium, caesium, strontium and barium - react rapidly, even dangerously to give the hydroxides. In part for this reason, alkali and alkaline earth metals are not found in nature in their metallic, i.e., native, form. Caesium is so reactive with oxygen that it is used as a getter in vacuum tubes, and solutions of potassium and sodium, so called NaK are used to deoxygenate and dehydrate some organic solvents. The surface of most metals consists of oxides and hydroxides in the presence of air. A well-known example is aluminum foil, which is coated with a thin film of aluminum oxide that passivates the metal, slowing further corrosion. The aluminum oxide layer can be built to greater thickness by the process of electrolytic anodizing. Although solid magnesium and aluminum react slowly with oxygen at STP, they, like most metals, will burn in air, generating very high temperatures. As a consequence, finely divided powders of most metals can be dangerously explosive in air.

OXIDIZED:
1. to convert (an element) into an oxide; combine with oxygen.
2. to cover with a coating of oxide or rust.
3. to take away hydrogen, as by the action of oxygen; add oxygen or any nonmetal.
4. to remove electrons from (an atom or molecule), thereby increasing the valence. Compare REDUCE (def. 12).

–verb (used without object)

5. to become oxidized.
6. (esp. of white wine) to lose freshness after prolonged exposure to air and often to darken in color.

OXIDIZING: The process of breaking down organic wastes into simpler elemental forms or by products. Also used to separate combined chlorine and convert it into free chlorine.

OXYGEN DEFICIENT ENVIRONMENT: One of the most dangerous threats to an operator upon entering a manhole.

OZONE: Ozone or trioxygen (O3) is a triatomic molecule, consisting of three oxygen atoms. It is an allotrope of oxygen that is much less stable than the diatomic O2. Ground-level ozone is an air pollutant with harmful effects on the respiratory systems of animals. Ozone in the upper atmosphere filters potentially damaging ultraviolet light from reaching the Earth's surface. It is present in low concentrations throughout the Earth's atmosphere. It has many industrial and consumer applications. Ozone, the first allotrope of a chemical element to be recognized by science, was proposed as a distinct chemical compound by Christian Friedrich Schönbein in 1840, who named it after the Greek word for smell (ozein), from the peculiar odor in lightning storms. The formula for ozone, O3, was not determined until 1865 by Jacques-Louis Soret and confirmed by Schönbein in 1867. Ozone is a powerful oxidizing agent, far better than dioxygen. It is also unstable at high concentrations, decaying to ordinary diatomic oxygen (in about half an hour in atmospheric conditions):
2 O3 = 3 O2
This reaction proceeds more rapidly with increasing temperature and decreasing pressure. Deflagration of ozone can be triggered by a spark, and can occur in ozone concentrations of 10 wt% or higher.

PACKING: Material, usually of woven fiber, placed in rings around the shaft of a pump and used to control the leakage from the stuffing box.

PAINT FILTER TEST: Test to determine free water content of sludge or dewatered solids sample. Usually used as the criteria for admission to a landfill.

PARAMECIUM: Paramecia are a group of unicellular ciliate protozoa formerly known as slipper animalcules from their slipper shape. They are commonly studied as a representative of the ciliate group. Simple cilia cover the body which allows the cell to move with a synchronous motion (like a caterpilla). There is also a deep oral groove containing inconspicuous compound oral cilia (as found in other peniculids) that is used to draw food inside. They generally feed upon bacteria and other small cells. Osmoregulation is carried out by a pair of contractile vacuoles, which actively expel water absorbed by osmosis from their surroundings. Paramecia are widespread in freshwater environments, and are especially common in scums. Paramecia are attracted by acidic conditions. Certain single-celled eukaryotes, such as Paramecium, are examples for exceptions to the universality of the genetic code (translation systems where a few codons differ from the standard ones).

PARTS PER MILLION (PPM): A common unit of measure used to express the number of parts of a substance contained within a million parts of a liquid, solid, or gas.

PASTEURIZATION: A process for killing pathogenic organisms by applying heat for a specific period of time.

PATHOGENS: Disease-causing pathogens; waterborne pathogens A pathogen may contaminate water and cause waterborne disease.

Pb: The chemical symbol of Lead.

PCE: abbr. perchloroethylene. Known also as perc or tetrachloroethylene, perchloroethylene is a clear, colorless liquid with a distinctive, somewhat ether-like odor. It is non-flammable, having no measurable flashpoint or flammable limits in air. Effective over a wide range of applications, perchloroethylene is supported by closed loop transfer systems, stabilizers and employee exposure monitoring.

pCi/L: Picocuries per liter A curie is the amount of radiation released by a set amount of a certain compound. A picocurie is one quadrillionth of a curie.

PEAK DEMAND: The maximum momentary load placed on a water treatment plant, pumping station or distribution system.

PERKINESIS: The aggregation resulting from random thermal motion of fluid molecules.

PERMEATE: The term for water which has passed through the membrane of a reverse osmosis unit. The liquid that passes through a membrane.

pH: A unit of measure which describes the degree of acidity or alkalinity of a solution. The pH scale runs from 0 to 14 with 7 being the mid-point or neutral. A pH of less than 7 is on the acid side of the scale with 0 as the point of greatest acid activity. A pH of more than 7 is on the basic (alkaline) side of the scale with 14 as the point of greatest basic activity. The term pH is derived from “p”, the mathematical symbol of the negative logarithm, and “H”, the chemical symbol of Hydrogen. The definition of pH is the negative logarithm of the Hydrogen ion activity. $pH=-\log[H^+]$.

pH OF SATURATION: The ideal pH for perfect water balance in relation to a particular total alkalinity level and a particular calcium hardness level, at a particular temperature. The pH where the Langelier Index equals zero.

PHENOLPHTHALEIN/TOTAL ALKALINITY: The relationship between the alkalinity constituent’s bicarbonate, carbonate, and hydroxide can be based on the P and T alkalinity measurement.

PHENOL RED: Chemical reagent used for testing pH in the range of 6.8 - 8.4.

PHOSPHATE, NITRATE AND ORGANIC NITROGEN: Nutrients in a domestic water supply reservoir may cause water quality problems if they occur in moderate or large quantities.

PHYSICAL CHEMICAL TREATMENT: Treatment processes that are non-biological in nature.

PICOCURIE: A unit of radioactivity. "Pico" is a metric prefix that means one one-millionth of one one-millionth. A picocurie is one one-millionth of one one-millionth of a Curie. A Curie is that quantity of any radioactive substance that undergoes 37 billion nuclear disintegrations per second. Thus a picocurie is that quantity of any radioactive substance that undergoes 0.037 nuclear disintegrations per second.

PIEZOMETRIC SURFACE: See potentiometric surface.

PIN FLOC: Small flocculated particle size.

PLATE AND FRAME PRESS: A batch process dewatering device in which sludge is pumped under high pressure through a series of parallel plates, in which a chamber is created between the plates. Each plate is fitted with filter cloth and the solids are collected in the chambers and the water is filtered from the sludge.

POINT SOURCE DISCHARGE: A pipe, ditch, channel or other container from which pollutants may be discharged.

POLLUTANT: A substance, organism or energy form present in amounts that impair or threaten an ecosystem to the extent that its current or future uses are prevented.

POLLUTION: To make something unclean or impure. See Contaminated.

POLYMER: A type of chemical when combined with other types of coagulants aid in binding small suspended particles to larger particles to help in the settling and filtering processes. Chemical used for flocculation in dewatering. Also known as a "polyelectrolyte" which is a substance made of giant molecules formed by the union of simple smaller molecules.

POLYPHOSPHATES: Chemicals that may be added to remove low levels of iron and manganese.

PORE SPACE: The interstitial space between sediments and fractures that is capable of storing and transmitting water.

POROSITY: A factor representing a rock, soil, or formations percentage of open space available for the percolation and storage of groundwater.

POSITIVE CONTROL: See Ongoing precision and recovery standard.

POST-CHLORINE: Where the water is chlorinated to make sure it holds a residual in the distribution system.

POST TREATMENT: Treatment of finished water or wastewater to further enhance its quality.

POTABLE: Good water which is safe for drinking or cooking purposes. Non-Potable: A liquid or water that is not approved for drinking.

POTENTIAL ENERGY: The energy that a body has by virtue of its position or state enabling it to do work.

PPM: Abbreviation for parts per million.

PRE-CHLORINE: Where the raw water is dosed with a large concentration of chlorine.

PRE-CHLORINATION: The addition of chlorine before the filtration process will help:
> Control algae and slime growth
> Control mud ball formation
> Improve coagulation
> Precipate iron
The addition of chlorine to the water prior to any other plant treatment processes.

PRECIPITATE: A solid that separates from a solution.

PRECIPTATION: The phenomenon that occurs when a substance held in solution passes out of solution into a solid form.

PRELIMINARY TREATMENT: Treatment steps including comminution, screening, grit removal, pre-aeration, and/or flow equalization that prepares wastewater influent for further treatment.

PRESSURE: Pressure is defined as force per unit area. It is usually more convenient to use pressure rather than force to describe the influences upon fluid behavior. The standard unit for pressure is the Pascal, which is a Newton per square meter. For an object sitting on a surface, the force pressing on the surface is the weight of the object, but in different orientations it might have a different area in contact with the surface and therefore exert a different pressure.

PRESSURE FILTER: Filter unit enclosed in a vessel that may be operated under pressure.

PRESSURE HEAD: The height of a column of water capable of being maintained by pressure. See also Total Head, Total Dynamic Head.

PRESSURE MEASUREMENT: Bourdon tube, Bellows gauge and Diaphragm are commonly used to measure pressure in waterworks systems. A Bellows-type sensor reacts to a change in pressure.

PREVENTION: To take action. Stop something before it happens.

PRIMARY CLARIFIER: Sedimentation basin that precedes secondary wastewater treatment.

PRIMARY SLUDGE: Sludge produced in a primary waste treatment unit.

PRIMARY TREATMENT: Treatment steps including sedimentation and/or fine screening to produce an effluent suitable for biological treatment.

PROCESS WASTEWATER: Wastewater generated during manufacture or production processes.

PROCESS WATER: Water that is used for, or comes in contact with an end product or the materials used in an end product.

PROPIONIC ACID: Rod-shaped, pleomorphic, gram-positive, anaerobic; ferment lactic acid; fermentation produces holes in Swiss cheese from the production of carbon dioxide.

PROTON, NEUTRON AND ELECTRON: Are the 3 fundamental particles of an atom.

PROTOZOA: Microscopic animals that occur as single cells. Some protozoa can cause disease in humans. Protozoa form cysts, which are specialized cells like eggs that are very resistant to chlorine. Cysts can survive the disinfection process, then "hatch" into normal cells that can cause disease. Protozoa must be removed from drinking water by filtration, because they cannot be effectively killed by chlorine.

PSEUDOMONAD: Rod-shaped (straight or curved) with polar flagella, gram-negative, aerobic; can use up to 100 different compounds for carbon and energy.

PUMPING LIFT: The height to which water must be pumped or lifted to, feet of head.

PTFE: Polytetrafluoroethylene.

QUANTITATIVE TRANSFER: The process of transferring a solution from one container to another using a pipette in which as much solution as possible is transferred, followed by rinsing of the walls of the source container with a small volume of rinsing solution (e.g., reagent water, buffer, etc.), followed by transfer of the rinsing solution, followed by a second rinse and transfer.

QUICKLIME: A calcium oxide material produced by calcining limestone to liberate carbon dioxide, also called “calcined lime” or “pebble lime”, commonly used for pH adjustment. Chemical formula is CaO.

RAW TURBIDITY: The turbidity of the water coming to the treatment plant from the raw water source.

REAGENT: A substance used in a chemical reaction to measure, detect, examine, or produce other substances.

REDOX POTENTIAL: Reduction potential (also known as redox potential, oxidation / reduction potential or ORP) is the tendency of a chemical species to acquire electrons and thereby be reduced. Each species has its own intrinsic reduction potential; the more positive the potential, the greater the species' affinity for electrons and tendency to be reduced. In aqueous solutions, the reduction potential is the tendency of the solution to either gain or lose electrons when it is subject to change by introduction of a new species. A solution with a higher (more positive) reduction potential than the new species will have a tendency to gain electrons from the new species (i.e. to be reduced by oxidizing the new species) and a solution with a lower (more negative) reduction potential will have a tendency to lose electrons to the new species (i.e. to be oxidized by reducing the new species). Just as the transfer of hydrogen ions between chemical species determines the pH of an aqueous solution, the transfer of electrons between chemical species determines the reduction potential of an aqueous solution. Like pH, the reduction potential represents an intensity factor. It does not characterize the capacity of the system for oxidation or reduction, in much the same way that pH does not characterize the buffering capacity.

RELAY LOGIC: The name of a popular method of automatically controlling a pump, valve, chemical feeder, and other devices.

RESERVOIR: An impoundment used to store water.

RICKETTSIA: Spherical or rod-shaped, gram-negative, aerobic; cause Rocky Mountain spotted fever and typhus; closely related to Agrobacterium, a common gall-causing plant bacterium.

ROTIFER: Rotifers get their name (derived from Greek and meaning "wheel-bearer"; they have also been called wheel animalcules) from the corona, which is composed of several ciliated tufts around the mouth that in motion resemble a wheel. These create a current that sweeps food into the mouth, where it is chewed up by a characteristic pharynx (called the mastax) containing a tiny, calcified, jaw-like structure called the trophi. The cilia also pull the animal, when unattached, through the water. Most free-living forms have pairs of posterior toes to anchor themselves while feeding. Rotifers have bilateral symmetry and a variety of different shapes. There is a well-developed cuticle which may be thick and rigid, giving the animal a box-like shape, or flexible, giving the animal a worm-like shape; such rotifers are respectively called loricate and illoricate.

RAW SEWAGE: Untreated wastewater and its contents.

RAW SLUDGE: Undigested sludge recently removed from a sedimentation basin.

RAW WATER: Untreated surface or groundwater.

REAGENT WATER BLANK: see Method blank.

REAGENT WATER: Water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte.

RECLAIMED WATER: Wastewater that has been treated to a level that allows for its reuse for a beneficial purpose.

RECLAMATION: The process of improving or restoring the condition of land or other material to a better or more useful state.

RECYCLING: The process by which recovered materials are transformed into new products.

RELATIVE STANDARD DEVIATION (RSD): The standard deviation divided by the mean times 100.

RESIDENCE TIME: The period of time that a volume of liquid remains in a tank or system.

RESPIRATION: Intake of oxygen and discharge of carbon dioxide as a result of biological oxidation.

RETURN ACTIVATED SLUDGE: Settled activated sludge that is returned to mix with raw or primary settled wastewater.

ROBERT HOOKE: Coined the term "cell" to describe the structures he saw while examining a piece of cork using a microscope.

ROTARY DRUM SCREEN: Cylindrical screen used to remove floatable and suspended solids.

RSD: See Relative standard deviation.

SANITARY SURVEY: Persons trained in public health engineering and the epidemiology of waterborne diseases should conduct the sanitary survey. The importance of a detailed sanitary survey of a new water source cannot be overemphasized. An on-site review of the water sources, facilities, equipment, operation, and maintenance of a public water systems for the purpose of evaluating the adequacy of the facilities for producing and distributing safe drinking water. The purpose of a non-regulatory sanitary survey is to identify possible biological and chemical pollutants which might affect a water supply.

SANITIZER: A disinfectant or chemical which disinfects (kills bacteria), kills algae and oxidizes organic matter.

SATURATION INDEX: See Langelier's Index.

SATURATOR: A device which produces a fluoride solution for the fluoride process. Crystal-grade types of sodium fluoride should be fed with a saturator. Overfeeding must be prevented to protect public health when using a fluoridation system.

SATURATED ZONE: Where an unconfined aquifer becomes saturated beneath the capillary fringe.

SCADA: A remote method of monitoring pumps and equipment. 130 degrees F is the maximum temperature that transmitting equipment is able to with stand. If the level controller may be set with too close a tolerance 45 could be the cause of a control system that is frequently turning a pump on and off.

SCALE: Crust of calcium carbonate, the result of unbalanced water. Hard insoluble minerals deposited (usually calcium bicarbonate) which forms on pool and spa surfaces and clog filters, heaters and pumps. Scale is caused by high calcium hardness and/or high pH. The regular use of stain prevention chemicals can prevent scale.

SCREENINGS PRESS: A mechanical press used to compact and/or dewater material removed from mechanical screening equipment.

SCROLL AND BASKET: The two basic types of centrifuges used in water treatment.

SCRUBBER: A device used to removal particulates or pollutant gases from combustion or chemical process exhaust streams.

SCUM: Floatable materials found on the surface of primary and secondary settling tanks consisting of food wastes, grease, fats, paper, foam, and similar matter.

SECONDARY CLARIFIER: A clarifier following a secondary treatment process, designed for gravity removal of suspended matter.

SECONDARY SLUDGE: The sludge from the secondary clarifier in a wastewater treatment plant.

SECONDARY TREATMENT: The treatment of wastewater through biological oxidation after primary treatment.

SEDIMENTATION: The removal of settleable suspended solids from water or wastewater by gravity in a quiescent basin or clarifier.

SEDIMENTATION BASIN: A quiescent tank used to remove suspended solids by gravity settling. Also called clarifiers or settling tanks, they are usually equipped with a motor driven rake mechanism to collect settled sludge and move it to a central discharge point.

SEDIMENTATION BASIN: Where the thickest and greatest concentration of sludge will be found. Twice a year sedimentation tanks should be drained and cleaned if the sludge buildup interferes with the treatment process.

SEDIMENTATION: The process of suspended solid particles settling out (going to the bottom of the vessel) in water.

SEDIMENT: Grains of soil, sand, gravel, or rock deposited by and generated by water movement.

SENSOR: A float and cable system are commonly found instruments that may be used as a sensor to control the level of liquid in a tank or basin.

SEPTIC: Condition characterized by bacterial decomposition under anaerobic conditions.

SETTLEABILITY: The tendency of suspended solids to settle.

SETTLEABLE SOLIDS: That portion of suspended solids which are of a sufficient size and weight to settle to the bottom of an Imhoff cone in one hour.

SETTLED SLUDGE VOLUME: Volume of settled sludge measured at predetermined time increments for use in process control calculations.

SETTLED SOLIDS: Solids that have been removed from the raw water by the coagulation and settling processes.

SEWAGE: Liquid or waterborne wastes polluted or fouled from households, commercial or industrial operations, along with any surface water, storm water or groundwater infiltration.

SEWER GAS: A gas mixture produced by anaerobic decomposition of organic matter usually containing high percentages of methane and hydrogen sulfide.

SHEATHED: Filamentous, gram-negative, aerobic; “swarmer” (colonizing) cells form and break out of a sheath; sometimes coated with metals from environment.

SHOCK: Also known as superchlorination or break point chlorination. Ridding a water of organic waste through oxidization by the addition of significant quantities of a halogen.

SHOCK LOAD: A sudden hydraulic or organic load to a treatment plant, also descriptive of a change in the material being treated.

SHORT-CIRCUITING: Short Circuiting is a condition that occurs in tanks or basins when some of the water travels faster than the rest of the flowing water. This is usually undesirable since it may result in shorter contact, reaction or settling times in comparison with the presumed detention times.

SHOULD: This action, activity, or procedural step is suggested but not required.

SINGLE PHASE POWER: The type of power used for lighting systems, small motors, appliances, portable power tools and in homes.

SLOP OIL: Separator skimmings and tramp oil generated during refinery startup, shutdown or abnormal operation.

SLUDGE: Accumulated and concentrated solids generated within a treatment process that have not undergone a stabilization process.

SLUDGE BASINS: After cleaning sludge basins and before returning the tanks into service the tanks should be inspected, repaired if necessary, and disinfected.

SLUDGE BLANKET: The accumulated sludge suspended in a clarifier or other enclosed body of water.

SLUDGE DEWATERING: The removal of a portion or majority of the water contained in sludge by means of a filter press, centrifuge or other mechanism.

SLUDGE DRYING BED: A closed area consisting of sand or other porous material upon which sludge is dewatered by gravity drainage and evaporation.

SLUDGE REDUCTION: Organic polymers are used to reduce the quantity of sludge. If a plant produces a large volume of sludge, the sludge could be dewatered, thickened, or conditioned to decrease the volume of sludge. Turbidity of source water, dosage, and type of coagulant used are the most important factors which determine the amount of sludge produced in a treatment of water.

SLURRY: A mixture of a solid and a liquid that facilitates the transfer of the solid into a treatment solution.

SOC: A common way for a synthetic organic chemical such as dioxin to be introduced to a surface water supply is from an industrial discharge, agricultural drainage, or a spill.

SODA ASH: Chemical used to raise pH and total alkalinity (sodium carbonate)

SODIUM BICARBONATE: Commonly used to increase alkalinity of water and stabilize pH.

SODIUM BISULFATE: Chemical used to lower pH and total alkalinity (dry acid).

SODIUM HYDROXIDE: Also known as caustic soda, a by-product chlorine generation and often used to raise pH.

SOFTENING WATER: When the water has a low alkalinity it is advantageous to use soda ash instead of caustic soda for softening water.

SOFTENING: The process that removes the ions which cause hardness in water.

SOLID, LIQUID AND VAPOR: 3 forms of matter.

SOLID WASTE: Garbage, refuse, sludge and other discarded material resulting from community activities or commercial or industrial operations.

SOLUBILITY: The amount of a substance that can dissolve in a solution under a given set of conditions.

SPADNS: The lab reagent called SPADNS solution is used in performing the Fluoride test.

SPIKING SUSPENSION: Diluted stock suspension containing the organism(s) of interest at a concentration appropriate for spiking samples.

SPIRILLUM: Spiral-shaped, gram-negative, aerobic; include Bdellovibrio, predatory on other bacteria.

SPIROCHETE: Spiral-shaped, gram-negative, mostly anaerobic; common in moist environments, from mammalian gums to coastal mudflats; complex internal structures convey rapid movement; include *Treponemapallidum,* cause of syphilis.

SPOROZOITE: A motile, infective stage of certain protozoans; e.g., *Cryptosporidium*. There are four sporozoites in each *Cryptosporidium* oocyst, and they are generally banana-shaped.

SPRAY BOTTLE OF AMMONIA: An operator should use ammonia to test for a chlorine leak around a valve or pipe. You will see white smoke if there is a leak.

SPRING PRESSURE: Is what maintains contact between the two surfaces of a mechanical seal.

STABILIZATION POND: A large shallow basin used for wastewater treatment by natural processes involving the use of algae and bacteria to accomplish biological oxidation of organic matter.

STERILIZED GLASSWARE: The only type of glassware that should be used in testing for coliform bacteria.

STOCK SUSPENSION: A concentrated suspension containing the organism(s) of interest that is obtained from a source that will attest to the host source, purity, authenticity, and viability of the organism(s).

STUFFING BOX: That portion of the pump that houses the packing or mechanical seal.

SUBNATANT: Liquid remaining beneath the surface of floating solids.

SUCCESSION: Transition in the species composition of a biological community, often following ecological disturbance of the community; the establishment of a biological community in an area virtually barren of life.

SULFIDE: The term sulfide refers to several types of chemical compounds containing sulfur in its lowest oxidation number of -2. Formally, "sulfide" is the dianion, S2-, which exists in strongly alkaline aqueous solutions formed from H2S or alkali metal salts such as Li2S, Na2S, and K2S. Sulfide is exceptionally basic and, with a pKa > 14, it does not exist in appreciable concentrations even in highly alkaline water, being undetectable at pH < ~15 (8 M NaOH). Instead, sulfide combines with electrons in hydrogen to form HS, which is variously called hydrogen sulfide ion, hydrosulfide ion, sulfhydryl ion, or bisulfide ion. At still lower pH's (<7), HS- converts to H2S, hydrogen sulfide. Thus, the exact sulfur species obtained upon dissolving sulfide salts depends on the pH of the final solution. Aqueous solutions of transition metals cations react with sulfide sources (H2S, NaSH, Na2S) to precipitate solid sulfides. Such inorganic sulfides typically have very low solubility in water and many are related to minerals. One famous example is the bright yellow species CdS or "cadmium yellow". The black tarnish formed on sterling silver is Ag2S. Such species are sometimes referred to as salts. In fact, the bonding in transition metal sulfides is highly covalent, which gives rise to their semiconductor properties, which in turn is related to the practical applications of many sulfide materials.

SULFATE- AND SULFUR- REDUCING: Commonly rod-shaped, mostly gram-negative, anaerobic; include *Desulfovibrio,* ecologically important in marshes.

SULFUR- AND IRON- OXIDIZING: Commonly rod-shaped, frequently with polar flagella, gram-negative, mostly anaerobic; most live in neutral (nonacidic) environment.

SUPERNATANT: The liquid layer which forms above the sludge in a settling basin.

SURFACE SEAL: The upper portion of a wells construction where surface contaminants are adequately prevented from entering the well, normally consisting of surface casing and neat cement grout.

SURFACTANT: Surfactants reduce the surface tension of water by adsorbing at the liquid-gas interface. They also reduce the interfacial tension between oil and water by adsorbing at the liquid-liquid interface. Many surfactants can also assemble in the bulk solution into aggregates. Examples of such aggregates are vesicles and micelles. The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. When micelles form in water, their tails form a core that can encapsulate an oil droplet, and their (ionic/polar) heads form an outer shell that maintains favorable contact with water. When surfactants assemble in oil, the aggregate is referred to as a reverse micelle. In a reverse micelle, the heads are in the core and the tails maintain favorable contact with oil. Surfactants are also often classified into four primary groups; anionic, cationic, non-ionic, and zwitterionic (dual charge).

SUSPENDED SOLIDS: Solids captured by filtration through a 0.45 micron filter membrane.

TCE, *trichloroethylene:* A solvent and degreaser used for many purposes; for example dry cleaning, it is a common groundwater contaminant. Trichloroethylene is a colorless liquid which is used as a solvent for cleaning metal parts. Drinking or breathing high levels of trichloroethylene may cause nervous system effects, liver and lung damage, abnormal heartbeat, coma, and possibly death. Trichloroethylene has been found in at least 852 of the 1,430 National Priorities List sites identified by the Environmental Protection Agency (EPA).

TDS-TOTAL DISSOLVED SOLIDS: An expression for the combined content of all inorganic and organic substances contained in a liquid which are present in a molecular, ionized or micro-granular (colloidal sol) suspended form. Generally, the operational definition is that the solids (often abbreviated TDS) must be small enough to survive filtration through a sieve size of two micrometers. Total dissolved solids are normally only discussed for freshwater systems, since salinity comprises some of the ions constituting the definition of TDS. The principal application of TDS is in the study of water quality for streams, rivers and lakes, although TDS is generally considered not as a primary pollutant (e.g. it is not deemed to be associated with health effects), but it is rather used as an indication of aesthetic characteristics of drinking water and as an aggregate indicator of presence of a broad array of chemical contaminants.

TDS: Ion exchange is an effective treatment process used to remove iron and manganese in a water supply. This process is ideal as long as the water does not contain a large amount of TDS. When determining the total dissolved solids, a sample should be filtered before being poured into an evaporating dish and dried. Demineralization may be necessary in a treatment process if the water has a very high value Total Dissolved Solids.

TELEMETERING: The use of a transmission line with remote signaling to monitor a pumping station or motors. Can be used to accomplish accurate and reliable remote monitoring and control over a long distribution system.

TEMPERATURE SAMPLE: This test should be performed immediately in the field, a grab sample.

TERTIARY TREATMENT: The use of physical, chemical, or biological means to improve secondary wastewater effluent quality.

THE RATE DECREASES: In general, when the temperature decreases, the chemical reaction rate decreases also.

THICKENING: A procedure used to increase the solids content of sludge by removing a portion of the liquid.

THICKENING, CONDITIONING AND DEWATERING: Common processes that are utilized to reduce the volume of sludge.

THOMAS MALTHUS: Formulated the concept that population growth proceeds at a geometric rate.

TIME FOR TURBIDITY BREAKTHROUGH AND MAXIMUM HEADLOSS: Are the two factors which determine whether or not a change in filter media size should be made.

TITRATION: A method of testing by adding a reagent of known strength to a water sample until a specific color change indicates the completion of the reaction.

TOTAL ALKALINITY: A measure of the acid-neutralizing capacity of water which indicates its buffering ability, i.e. measure of its resistance to a change in pH. Generally, the higher the total alkalinity, the greater the resistance to pH change.

TOTAL COLIFORM: Total coliform, fecal coliform, and E. coli are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. E. coli is a sub-group of fecal coliform. When a water sample is sent to a lab, it is tested for total coliform. If total coliform is present, the sample will also be tested for either fecal coliform or E. coli, depending on the lab testing method.

TOTAL DISSOLVED SOLIDS (TDS): The accumulated total of all solids that might be dissolved in water. The weight per unit volume of all volatile and non-volatile solids dissolved in a water or wastewater after a sample has been filtered to remove colloidal and suspended solids.

TOTAL DYNAMIC HEAD: The pressure (psi) or equivalent feet of water, required for a pump to lift water to its point of storage overcoming elevation head, friction loss, line pressure, drawdown and pumping lift.

TOTAL SOLIDS: The sum of dissolved and suspended solids in a water or wastewater.

TOTAL SUSPENDED SOLIDS: The measure of particulate matter suspended in a sample of water or wastewater.

TOXIC: Capable of causing an adverse effect on biological tissue following physical contact or absorption.

TREATABILITY STUDY: A study in which a waste is subjected to a treatment process to determine treatment and/or to determine the treatment efficiency or optimal process conditions for treatment.

TRIHALOMETHANES (THM): Four separate compounds including chloroform, dichlorobromomethane, dibromochloromethane, and bromoform. The most common class of disinfection by-products created when chemical disinfectants react with organic matter in water during the disinfection process. See Disinfectant Byproducts.

TUBE SETTLERS: This modification of the conventional process contains many metal tubes that are placed in the sedimentation basin, or clarifier. These tubes are approximately 1 inch deep and 36 inches long, split-hexagonal shape and installed at an angle of 60 degrees or less. These tubes provide for a very large surface area upon which particles may settle as the water flows upward. The slope of the tubes facilitates gravity settling of the solids to the bottom of the basin, where they can be collected and removed. The large surface settling area also means that adequate clarification can be obtained with detention times of 15 minutes or less. As with conventional treatment, this sedimentation step is followed by filtration through mixed media.

TUBERCLES: The creation of this condition is of the most concern regarding corrosive water effects on a water system. Tubercles are formed due to joining dissimilar metals, causing electro-chemical reactions. Like iron to copper pipe. We have all seen these little rust mounds inside cast iron pipe.

TURBIDIMETER: Monitoring the filter effluent turbidity on a continuous basis with an in-line instrument is a recommended practice. Turbidimeter is best suited to perform this measurement.

TURBIDITY: A measure of the cloudiness of water caused by suspended particles. A qualitative measurement of water clarity which results from suspended matter that scatters or otherwise interferes with the passage of light through the water.

ULTRAFILTRATION: A low pressure membrane filtration process which separates solutes up to 0.1 micron size range.

UNDER PRESSURE IN STEEL CONTAINERS: After chlorine gas is manufactured, it is primarily transported in steel containers.

UP FLOW CLARIFIER: Clarifier where flocculated water flows upward through a sludge blanket to obtain floc removal by contact with flocculated solids in the blanket.

U.S. ENVIRONMENTAL PROTECTION AGENCY: In the United States, this agency responsible for setting drinking water standards and for ensuring their enforcement. This agency sets federal regulations which all state and local agencies must enforce.

VANE: That portion of an impeller that throws the water toward the volute.

VAPOR: The gaseous phase of a material that is in the solid or liquid state at standard temperature and pressure.

VARIABLE DISPLACEMENT PUMP: A pump that will produce different volumes of water dependent on the pressure head against it.

VELOCITY HEAD: The vertical distance a liquid must fall to acquire the velocity with which it flows through the piping system. For a given quantity of flow, the velocity head will vary indirectly as the pipe diameter varies.

VENTURI: If water flows through a pipeline at a high velocity, the pressure in the pipeline is reduced. Velocities can be increased to a point that a partial vacuum is created.

VERTICAL TURBINE: A type of variable displacement pump in which the motor or drive head is mounted on the wellhead and rotates a drive shaft connected to the pump impellers.

VIBRIO: Rod- or comma-shaped, gram-negative, aerobic; commonly with a single flagellum; include *Vibrio cholerae,* cause of cholera, and luminescent forms symbiotic with deep-water fishes and squids.

VIRUSES: Very small disease-causing microorganisms that are too small to be seen even with microscopes. Viruses cannot multiply or produce disease outside of a living cell.

VITRIFICATION: Vitrification is a process of converting a material into a glass-like amorphous solid that is free from any crystalline structure, either by the quick removal or addition of heat, or by mixing with an additive. Solidification of a vitreous solid occurs at the glass transition temperature (which is lower than melting temperature, Tm, due to supercooling). When the starting material is solid, vitrification usually involves heating the substances to very high temperatures. Many ceramics are produced in such a manner. Vitrification may also occur naturally when lightning strikes sand, where the extreme and immediate heat can create hollow, branching rootlike structures of glass, called fulgurite. When applied to whiteware ceramics, vitreous means the material has an extremely low permeability to liquids, often but not always water, when determined by a specified test regime. The microstructure of whiteware ceramics frequently contain both amorphous and crystalline phases.

VOLATILE: A substance that evaporates or vaporizes at a relatively low temperature.

VOLATILE ORGANIC COMPOUNDS (VOCs): Solvents used as degreasers or cleaning agents. Improper disposal of VOCs can lead to contamination of natural waters. VOCs tend to evaporate very easily. This characteristic gives VOCs very distinct chemical odors like gasoline, kerosene, lighter fluid, or dry cleaning fluid. Some VOCs are suspected cancer-causing agents. Volatile organic compounds (VOCs) are organic chemical compounds that have high enough vapor pressures under normal conditions to significantly vaporize and enter the atmosphere. A wide range of carbon-based molecules, such as aldehydes, ketones, and other light hydrocarbons are VOCs. The term often is used in a legal or regulatory context and in such cases the precise definition is a matter of law. These definitions can be contradictory and may contain "loopholes"; e.g. exceptions, exemptions, and exclusions. The United States Environmental Protection Agency defines a VOC as any organic compound that participates in a photoreaction; others believe this definition is very broad and vague as organics that are not volatile in the sense that they vaporize under normal conditions can be considered volatile by this EPA definition. The term may refer both to well characterized organic compounds and to mixtures of variable composition.

VOID: An opening, gap, or space within rock or sedimentary formations formed at the time of origin or deposition.

VOLTAGE: Voltage (sometimes also called electric or electrical tension) is the difference of electrical potential between two points of an electrical or electronic circuit, expressed in volts.[1] It measures the potential energy of an electric field to cause an electric current in an electrical conductor. Depending on the difference of electrical potential it is called extra low voltage, low voltage, high voltage or extra high voltage. Specifically Voltage is equal to energy per unit charge.

VOLUTE: The spiral-shaped casing surrounding a pump impeller that collects the liquid discharge by the impeller.

VORTICELLA: Vorticella is a genus of protozoa, with over 100 known species. They are stalked inverted bell-shaped ciliates, placed among the peritrichs. Each cell has a separate stalk anchored onto the substrate, which contains a contracile fibril called a myoneme. When stimulated this shortens, causing the stalk to coil like a spring. Reproduction is by budding, where the cell undergoes longitudinal fission and only one daughter keeps the stalk. Vorticella mainly lives in freshwater ponds and streams - generally anywhere protists are plentiful. Other genera such as Carchesium resemble Vorticella but are branched or colonial.

VORTEX: The helical swirling of water moving towards a pump.

VIRUSES: are very small disease-causing microorganisms that are too small to be seen even with microscopes. Viruses cannot multiply or produce disease outside of a living cell.

VULNERABILITY ASSESSMENT: An evaluation of drinking water source quality and its vulnerability to contamination by pathogens and toxic chemicals.

WAIVERS: Monitoring waivers for nitrate and nitrite are prohibited.

WASTE ACTIVATED SLUDGE: Excess activated sludge that is discharged from an activated sludge treatment process.

WASTEWATER: Liquid or waterborne wastes polluted or fouled from households, commercial or industrial operations, along with any surface water, storm water or groundwater infiltration.

WATER HAMMER: A surge in a pipeline resulting from the rapid increase or decrease in water flow. Water hammer exerts tremendous force on a system and can be highly destructive.

WATER QUALITY CRITERIA: Comprised of both numeric and narrative criteria. Numeric criteria are scientifically derived ambient concentrations developed by EPA or States for various pollutants of concern to protect human health and aquatic life. Narrative criteria are statements that describe the desired water quality goal.

WATER QUALITY STANDARD: A statute or regulation that consists of the beneficial designated use or uses of a waterbody, the numeric and narrative water quality criteria that are necessary to protect the use or uses of that particular waterbody, and an antidegradation statement.

WATERBORNE DISEASE: A disease, caused by a virus, bacterium, protozoan, or other microorganism, capable of being transmitted by water (e.g., typhoid fever, cholera, amoebic dysentery, gastroenteritis).

WATER RECLAMATION: The restoration of wastewater to a state that will allow its beneficial reuse.

WHOLE EFFLUENT TOXICITY: The total toxic effect of an effluent measured directly with a toxicity test.

WPCF: Water Pollution Control Facility

WTP: Water Treatment Plant

WWTP: Wastewater Treatment Plant

ZERO DISCHARGE: A facility that discharges no liquid effluent to the environment.

Glossary References

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Foster, Laurence, M.D. 1985. “Waterborne Disease - It’s Our Job to Prevent It”. PIPELINE newsletter, Oregon Health Division, Drinking Water Program, Portland, Oregon 1(4): 1-3.

Foster, Laurence, M.D. 1990. “Waterborne *Disease,” Methods for the Investigation and Prevention of Waterborne Disease Outbreaks.* Ed. Gunther F. Craun. Cincinnati: U.S. Environmental Protection Agency.

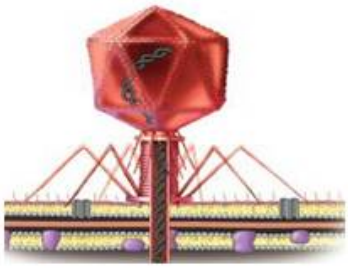
Operators analyze sludge samples to improve wasting.

Microorganism Appendix

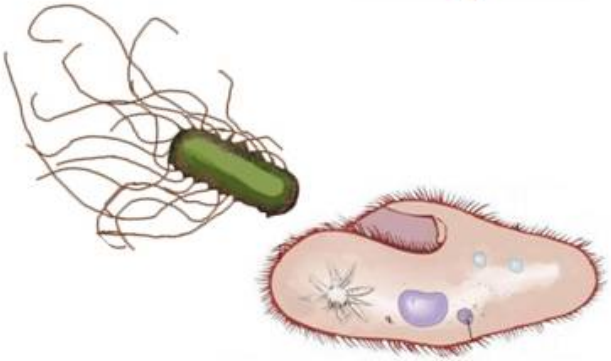
COCCI
(SPHERICAL)

SPIRILLI
(SPIRAL)

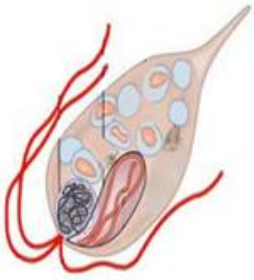
BACILLI
(ROD-SHAPED)



VIRUSES



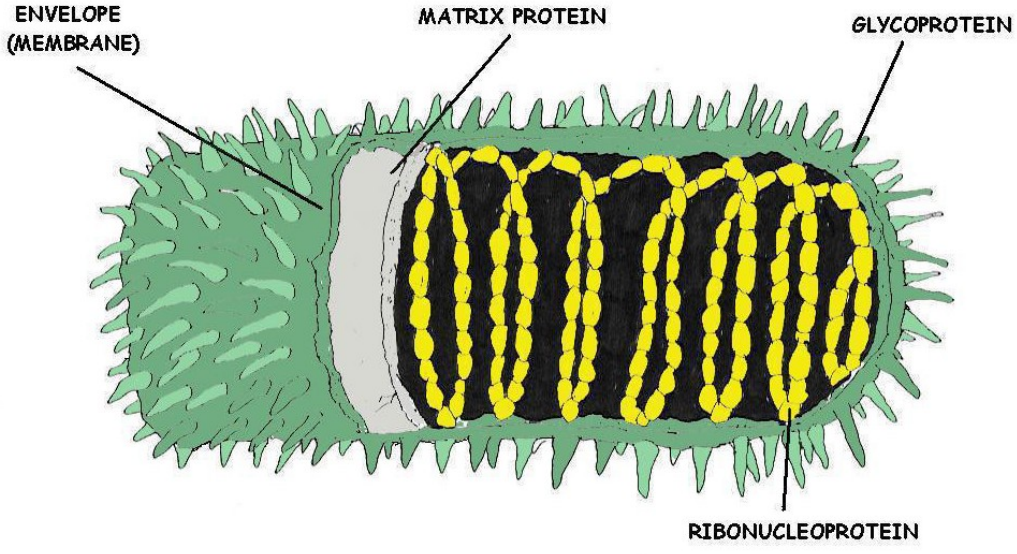
BACTERIA



PROTOZOA

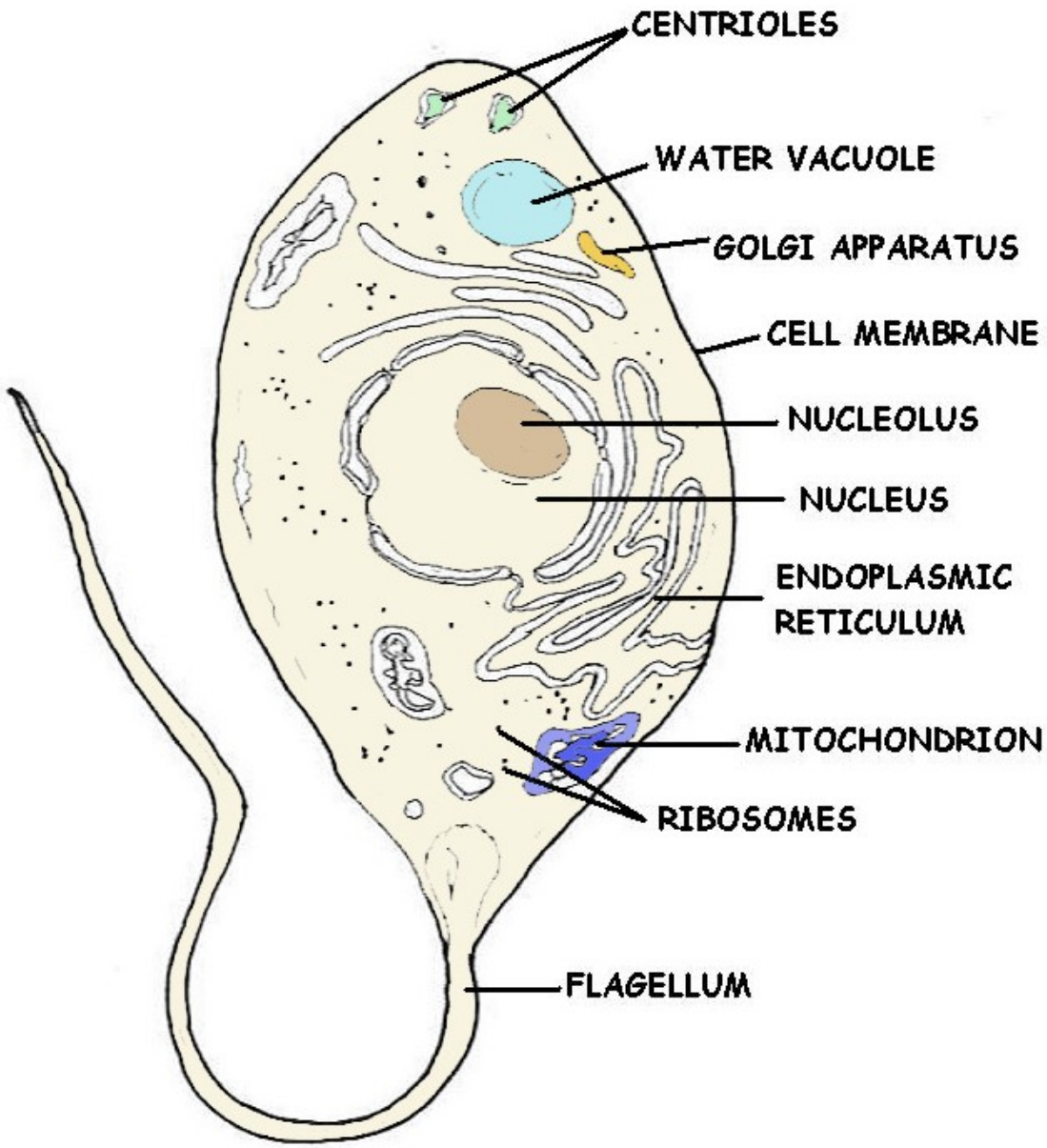
BACTERIA TYPES

This section will give a close-up and short explanation of the major microorganisms found in water and in wastewater.



VIRUS

Protozoa



PROTOZOAN CELL

Protozoa are around 10–50 micrometer, but can grow up to 1 mm and can easily be seen under a microscope. Protozoa exist throughout aqueous environments and soil. Protozoa occupy a range of trophic levels. As predators, they prey upon unicellular or filamentous algae, bacteria, and microfungi.

Protozoa play a role both as herbivores and as consumers in the decomposer link of the food chain. Protozoa also play a vital role in controlling bacteria populations and biomass. As components of the micro- and meiofauna, protozoa are an important food source for microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is important. Protozoa such as the malaria parasites (Plasmodium spp.), trypanosomes and leishmania are also important as parasites and symbionts of multicellular animals.

Most protozoa exist in 5 stages of life which are in the form of trophozoites and cysts. As cysts, protozoa can survive harsh conditions, such as exposure to extreme temperatures and harmful chemicals, or long periods without access to nutrients, water, or oxygen for a period of time. Being a cyst enables parasitic species to survive outside of the host, and allows their transmission from one host to another. When protozoa are in the form of trophozoites (Greek, tropho=to nourish), they actively feed and grow. The process by which the protozoa takes its cyst form is called encystation, while the process of transforming back into trophozoite is called excystation.

Protozoa can reproduce by binary fission or multiple fission. Some protozoa reproduce sexually, some asexually, and some both (e.g. Coccidia). An individual protozoan is hermaphroditic.

Classification
Protozoa were commonly grouped in the kingdom of Protista together with the plant-like algae and fungus-like water molds and slime molds. In the 21st-century systematics, protozoans, along with ciliates, mastigophorans, and apicomplexans, are arranged as animal-like protists. However, protozoans are neither Animalia nor Metazoa (with the possible exception of the enigmatic, moldy Myxozoa).

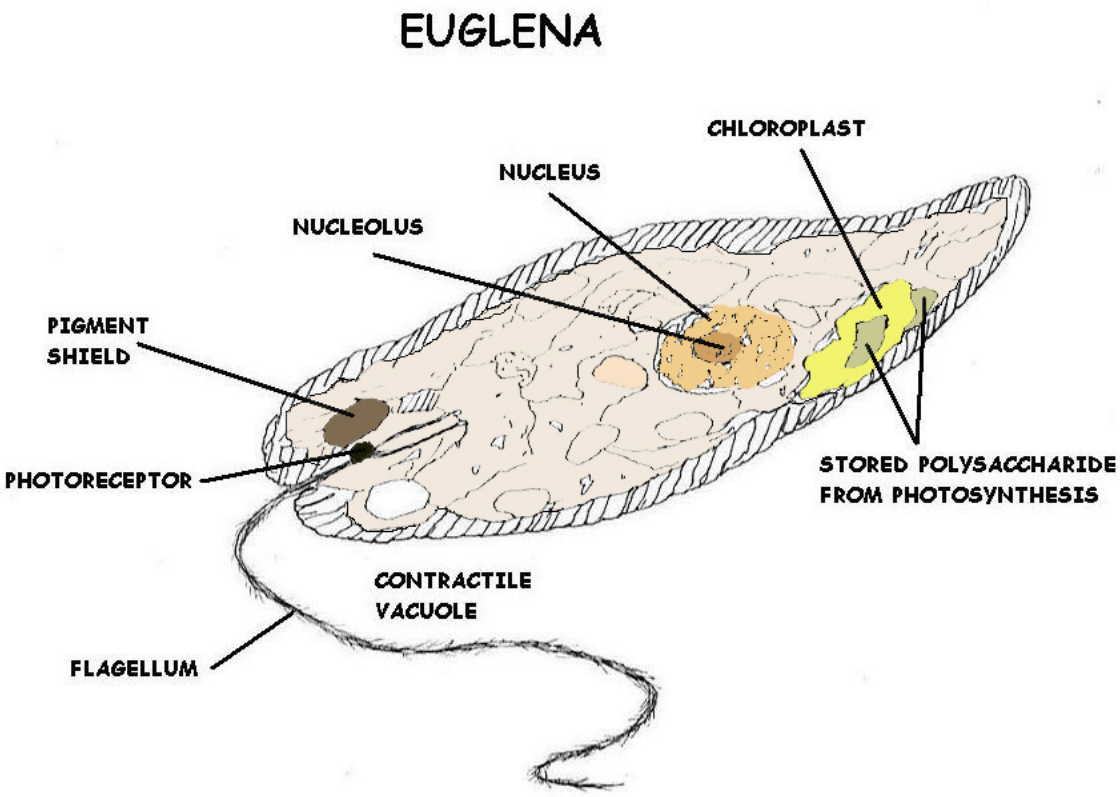
Sub-groups
Protozoa have traditionally been divided on the basis of their means of locomotion, although this is no longer believed to represent genuine relationships:
* Flagellates (e.g. Giardia lambia)
* Amoeboids (e.g. Entamoeba histolytica)
* Sporozoans (e.g. Plasmodium knowlesi)
* Apicomplexa
* Myxozoa
* Microsporidia
* Ciliates (e.g. Balantidium coli)

There are many ways that infectious diseases can spread. Pathogens usually have specific routes by which they are transmitted, and these routes may depend on the type of cells and tissue that a particular agent targets. For example, because cold viruses infect the respiratory tract, they are dispersed into the air via coughing and sneezing.

Once in the air, the viruses can infect another person who is unlucky enough to inhale air containing the virus particles.

Agents vary greatly in their stability in the environment. Some viruses may survive for only a few minutes outside of a host, while some spore-forming bacteria are extremely durable and may survive in a dormant state for a decade or more.

Protozoa Section



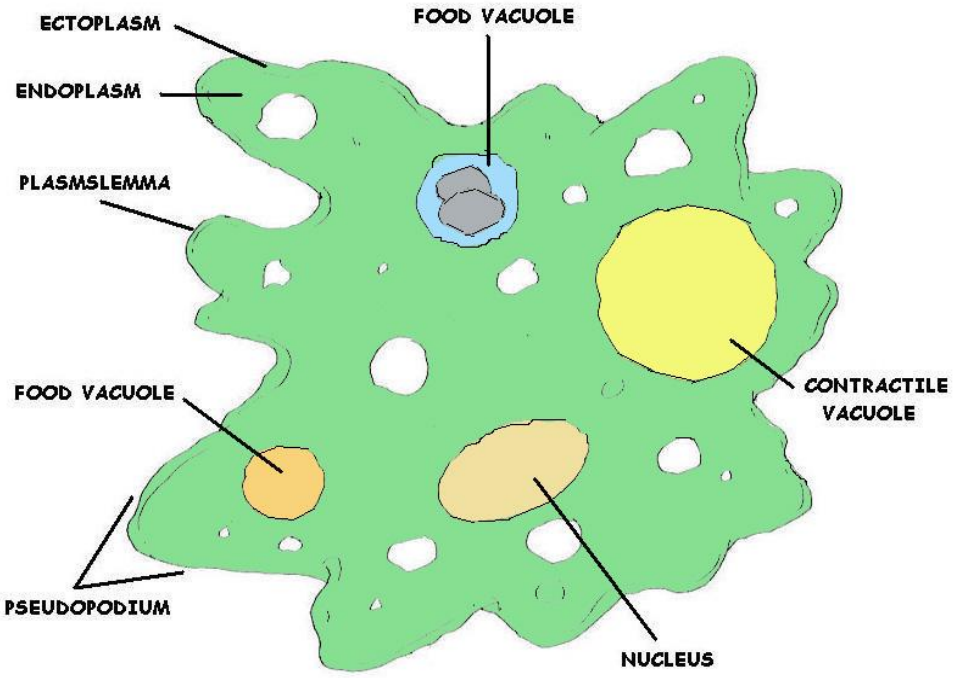
The diverse assemblage of organisms that carry out all of their life functions within the confines of a single, complex eukaryotic cell are called protozoa.

Paramecium, Euglena, and Amoeba are well-known examples of these major groups of organisms. Some protozoa are more closely related to animals, others to plants, and still others are relatively unique. Although it is not appropriate to group them together into a single taxonomic category, the research tools used to study any unicellular organism are usually the same, and the field of protozoology has been created to carry out this research. The unicellular photosynthetic protozoa are sometimes also called algae and are addressed elsewhere. This report considers the status of our knowledge of heterotrophic protozoa (protozoa that cannot produce their own food).

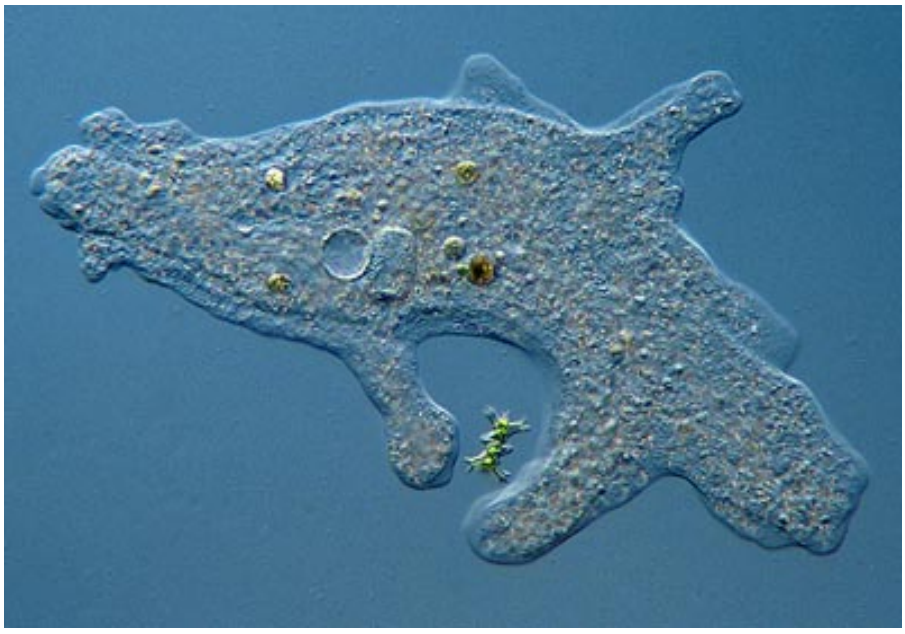
Free-living Protozoa

Protozoans are found in all moist habitats within the United States, but we know little about their specific geographic distribution. Because of their small size, production of resistant cysts, and ease of distribution from one place to another, many species appear to be cosmopolitan and may be collected in similar microhabitats worldwide (Cairns and Ruthven 1972). Other species may have relatively narrow limits to their distribution.

Marine ciliates inhabit interstices of sediment and beach sands, surfaces, deep sea and cold Antarctic environments, planktonic habitats, and the algal mats and detritus of estuaries and wetlands.



AMOEBA



Amoeba proteus*, pseudopods slowly engulf the small desmid *Staurastrum.

Amoebas

Amoebas (Phylum Rhizopoda) are unicellular protists that are able to change their shape constantly. Each species has its own distinct repertoire of shapes.

How does an amoeba locomote?
Amoebas locomote by way of cytoplasmic movement. (cytoplasm is the cell content around the nucleus of the cell) The amoeba forms pseudopods (false feet) with which they 'flow' over a surface. The cytoplasma not only flows, it also changes from a fluid into a solid state.

These pseudopods are also used to capture prey, they simply engulf the food. They can detect the kind of prey and use different 'engulfing tactics'.

The image from the last page shows several cell organelles. Left from the center we can see aspherical water expelling vesicle and just right of it, the single nucleus of this species can be seen. Other species may have many nuclei. The cell is full of brown food vacuoles and also contains small crystals.

Protozoa Information
Our actual knowledge of salinity, temperature, and oxygen requirements of marine protozoa is poor (although some groups, such as the foraminifera, are better studied than others), and even the broadest outlines of their biogeographic ranges are usually a mystery. In general, freshwater protozoan communities are similar to marine communities except the specialized interstitial fauna of the sand is largely missing. In freshwater habitats, the foraminifera and radiolaria common in marine environments are absent or low in numbers while testate amoebae exist in greater numbers. Relative abundance of species in the marine versus freshwater habitat is unknown.

Soil-dwelling protozoa have been documented from almost every type of soil and in every kind of environment, from the peat-rich soil of bogs to the dry sands of deserts. In general, protozoa are found in greatest abundance near the soil surface, especially in the upper 15 cm (6 in), but occasional isolates can be obtained at depths of a meter (yard) or more.

Protozoa do not constitute a major part of soil biomass, but in some highly productive regions such as forest litter, the protozoa are a significant food source for the microinvertebrates, with a biomass that may reach 20 g/m2 of soil surface area there.

Environmental Quality Indicators
Polluted waters often have a rich and characteristic protozoan fauna. The relative abundance and diversity of protozoa are used as indicators of organic and toxic pollution (Cairns et al. 1972; Foissner 1987; Niederlehner et al. 1990; Curds 1992). Bick (1972), for example, provided a guide to ciliates that are useful as indicators of environmental quality of European freshwater systems, along with their ecological distribution with respect to parameters such as amount of organic material and oxygen levels.

Foissner (1988) clarified the taxonomy of European ciliates as part of a system for classifying the state of aquatic habitats according to their faunas.

Symbiotic Protozoa
Parasites
Protozoa are infamous for their role in causing disease, and parasitic species are among the best-known protozoa. Nevertheless, our knowledge has large gaps, especially of normally free-living protozoa that may become pathogenic in immunocompromised individuals. For example, microsporidia comprise a unique group of obligate, intracellular parasitic protozoa. Microsporidia are amazingly diverse organisms with more than 700 species and 80 genera that are capable of infecting a variety of plant, animal, and even other protist hosts.

They are found worldwide and have the ability to thrive in many ecological conditions. Until the past few years, their ubiquity did not cause a threat to human health, and few systematists worked to describe and classify the species. Since 1985, however, physicians have documented an unusual rise in worldwide infections in AIDS patients caused by four different genera of microsporidia (Encephalitozoon, Nosema, Pleistophora, and Enterocytozoon). According to the Centers for Disease Control in the United States, difficulties in identifying microsporidian species are impeding diagnosis and effective treatment of AIDS patients.

Protozoan Reservoirs of Disease
The presence of bacteria in the cytoplasm of protozoa is well known, whereas that of viruses is less frequently reported. Most of these reports simply record the presence of bacteria or viruses and assume some sort of symbiotic relationship between them and the protozoa. Recently, however, certain human pathogens were shown to not only survive but also to multiply in the cytoplasm of free-living, nonpathogenic protozoa. Indeed, it is now believed that protozoa are the natural habitat for certain pathogenic bacteria. To date, the main focus of attention has been on the bacterium Legionella pneumophila, the causative organism of Legionnaires' disease; these bacteria live and reproduce in the cytoplasm of some free-living amoebae (Curds 1992). More on this subject in the following chapters.

Symbionts
Some protozoa are harmless or even beneficial symbionts. A bewildering array of ciliates, for example, inhabit the rumen and reticulum of ruminates and the cecum and colon of equids. Little is known about the relationship of the ciliates to their host, but a few may aid the animal in digesting cellulose.

Data on Protozoa
While our knowledge of recent and fossil foraminifera in the U.S. coastal waterways is systematically growing, other free-living protozoa are poorly known. There are some regional guides and, while some are excellent, many are limited in scope, vague on specifics, or difficult to use. Largely because of these problems, most ecologists who include protozoa in their studies of aquatic habitats do not identify them, even if they do count and measure them for biomass estimates (Taylor and Sanders 1991).

Parasitic protozoa of humans, domestic animals, and wildlife are better known although no attempt has been made to compile this information into a single source. Large gaps in our knowledge exist, especially for haemogregarines, microsporidians, and myxosporidians (see Kreier and Baker 1987).

Museum Specimens
For many plant and animal taxa, museums represent a massive information resource. This is not true for protozoa. In the United States, only the National Natural History Museum (Smithsonian Institution) has a reference collection preserved on microscope slides, but it does not have a protozoologist curator and cannot provide species' identification or verification services. The American Type Culture Collection has some protozoa in culture, but its collection includes relatively few kinds of protozoa.

Ecological Role of Protozoa
Although protozoa are frequently overlooked, they play an important role in many communities where they occupy a range of trophic levels. As predators upon unicellular or filamentous algae, bacteria, and microfungi, protozoa play a role both as herbivores and as consumers in the decomposer link of the food chain. As components of the micro- and meiofauna, protozoa are an important food source for microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is important.

Factors Affecting Growth and Distribution
Most free-living protozoa reproduce by cell division (exchange of genetic material is a separate process and is not involved in reproduction in protozoa). The relative importance for population growth of biotic versus chemical-physical components of the environment is difficult to ascertain from the existing survey data. Protozoa are found living actively in nutrient-poor to organically rich waters and in fresh water varying between 0°C (32°F) and 50°C (122°F). Nonetheless, it appears that rates of population growth increase when food is not constrained and temperature is increased (Lee and Fenchel 1972; Fenchel 1974; Montagnes et al. 1988).

Comparisons of oxygen consumption in various taxonomic groups show wide variation (Laybourn and Finlay 1976), with some aerobic forms able to function at extremely low oxygen tensions and to thereby avoid competition and predation.

Many parasitic and a few free-living species are obligatory anaerobes (grow without atmospheric oxygen). Of the free-living forms, the best known are the plagiopylid ciliates that live in the anaerobic sulfide-rich sediments of marine wetlands (Fenchel et al. 1977). The importance of plagiopylids in recycling nutrients to aerobic zones of wetlands is potentially great.

Because of the small size of protozoa, their short generation time, and (for some species) ease of maintaining them in the laboratory, ecologists have used protozoan populations and communities to investigate competition and predation.

The result has been an extensive literature on a few species studied primarily under laboratory conditions. Few studies have been extended to natural habitats with the result that we know relatively little about most protozoa and their roles in natural communities. Intraspecific competition for common resources often results in cannibalism, sometimes with dramatic changes in morphology of the cannibals (Giese 1973). Field studies of interspecific competition are few and most evidence for such species interactions is indirect (Cairns and Yongue 1977).

Contractile Vacuoles

Many protozoa have contractile vacuoles, which collect and expel excess water, and extrusomes, which expel material used to deflect predators or capture prey. In multicellular organisms, hormones are often produced in vesicles. In higher plants, most of a cell's volume is taken up by a central vacuole or tonoplast, which maintains its osmotic pressure. Many eukaryotes have slender motile projections, usually called flagella when long and cilia when short. These are variously involved in movement, feeding, and sensation. These are entirely distinct from prokaryotic flagella. They are supported by a bundle of microtubules arising from a basal body, also called a kinetosome or centriole, characteristically arranged as nine doublets surrounding two singlets. Flagella also may have hairs or mastigonemes, scales, connecting membranes, and internal rods. Their interior is continuous with the cell's cytoplasm.

Centrioles

Centrioles are often present even in cells and groups that do not have flagella. They generally occur in groups of one or two, called kinetids that give rise to various microtubular roots. These form a primary component of the cytoskeletal structure, and are often assembled over the course of several cell divisions, with one flagellum retained from the parent and the other derived from it. Centrioles may also be associated in the formation of a spindle during nuclear division. Some protists have various other microtubule-supported organelles. These include the radiolaria and heliozoa, which produce axopodia used in flotation or to capture prey, and the haptophytes, which have a peculiar flagellum-like organelle called the haptonema.

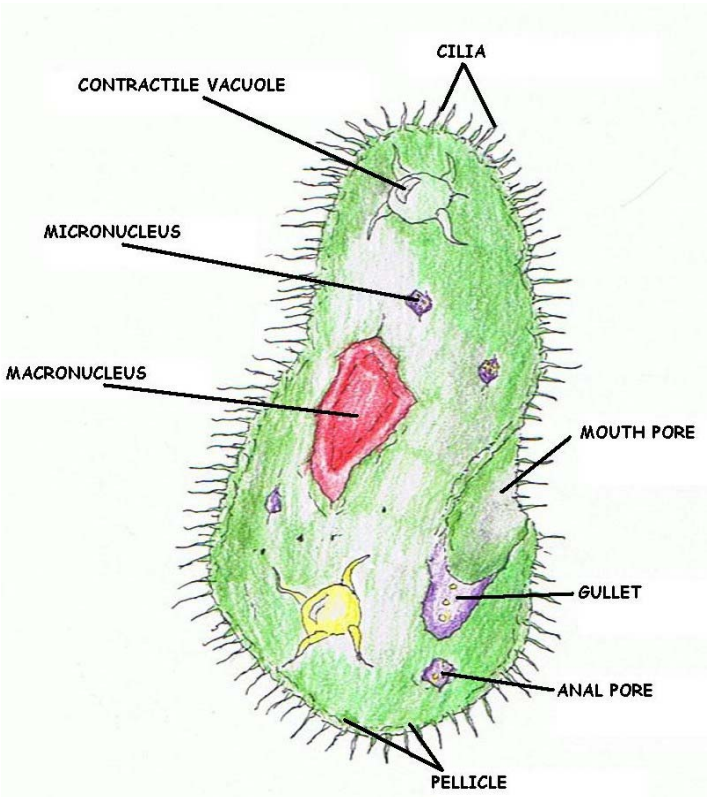
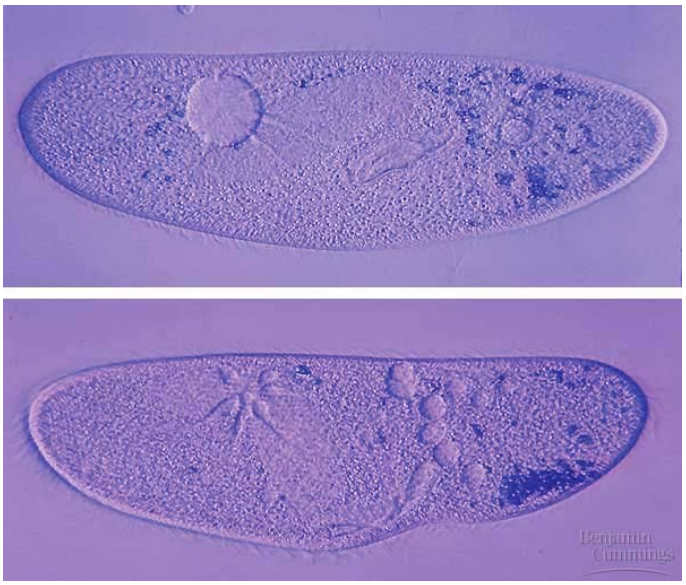


Figure 1. A diagram of *Paramecium* sp. with major organelles indicated.



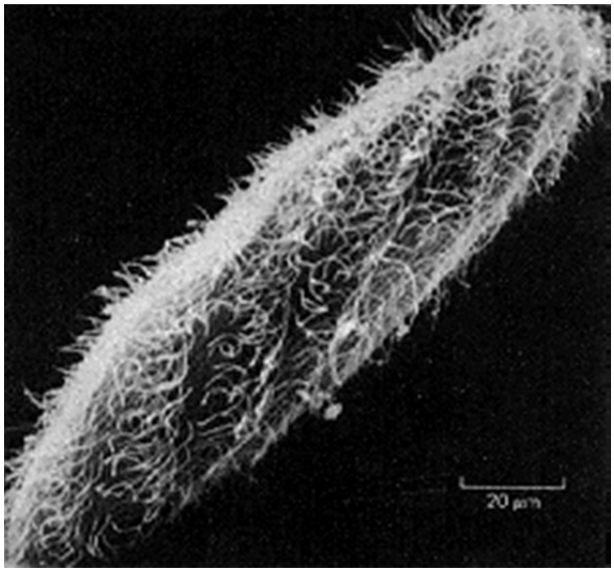
Contractile Vacuoles

Figure 2. The contractile vacuole when full (top) and after contraction (bottom).

Paramecium

Members of the genus *Paramecium* are single-celled, freshwater organisms in the kingdom Protista. They exist in an environment in which the osmotic concentration in their external environment is much lower than that in their cytoplasm. More specifically, the habitat in which they live is **hypotonic** to their cytoplasm. As a result of this, *Paramecium* is subjected to a continuous influx of water, as water diffuses inward to a region of higher osmotic concentration.

If *Paramecium* is to maintain homeostasis, water must be continually pumped out of the cell (against the osmotic gradient) at the same rate at which it moves in. This process, known as **osmoregulation**, is carried out by two organelles in *Paramecium* known as **contractile vacuoles** (Figures 1 and 2).



Here is a wastewater Inspector is utilizing the auto sampler's manual to help adjust the time and adjust for the correct flow for a composite sample. You can see that she is pouring off the pickle jar. This inspector is also the course author.

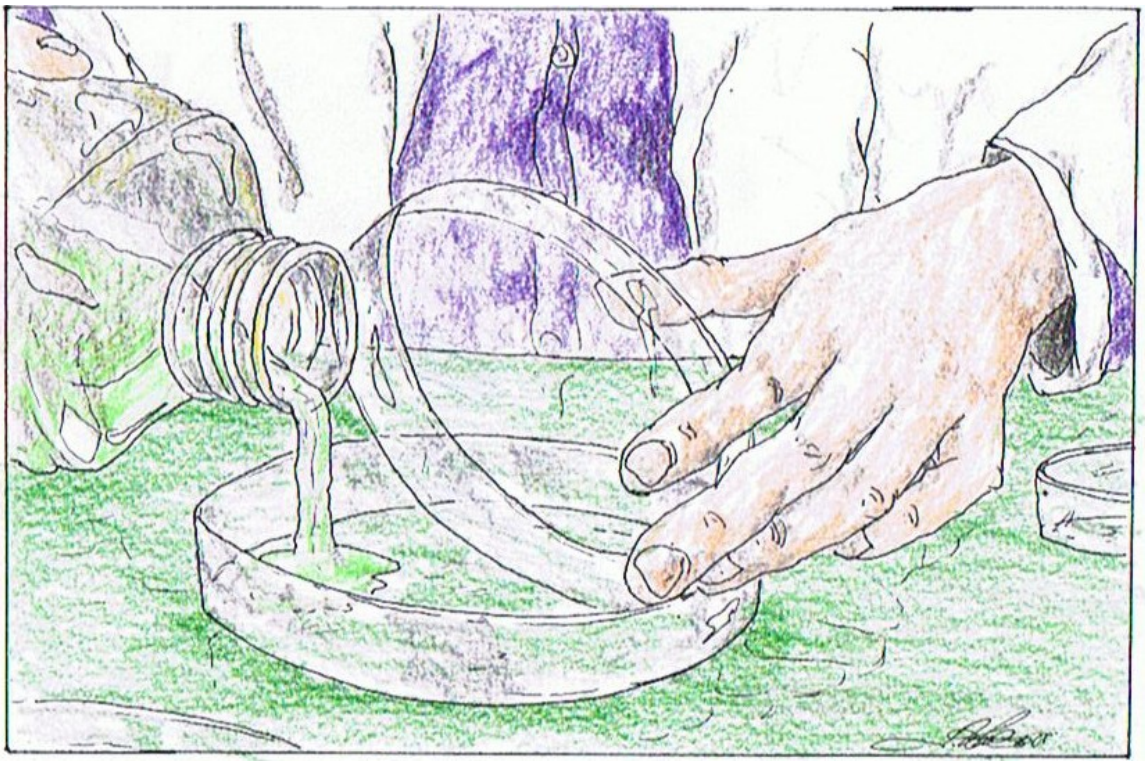
Protozoan Diseases

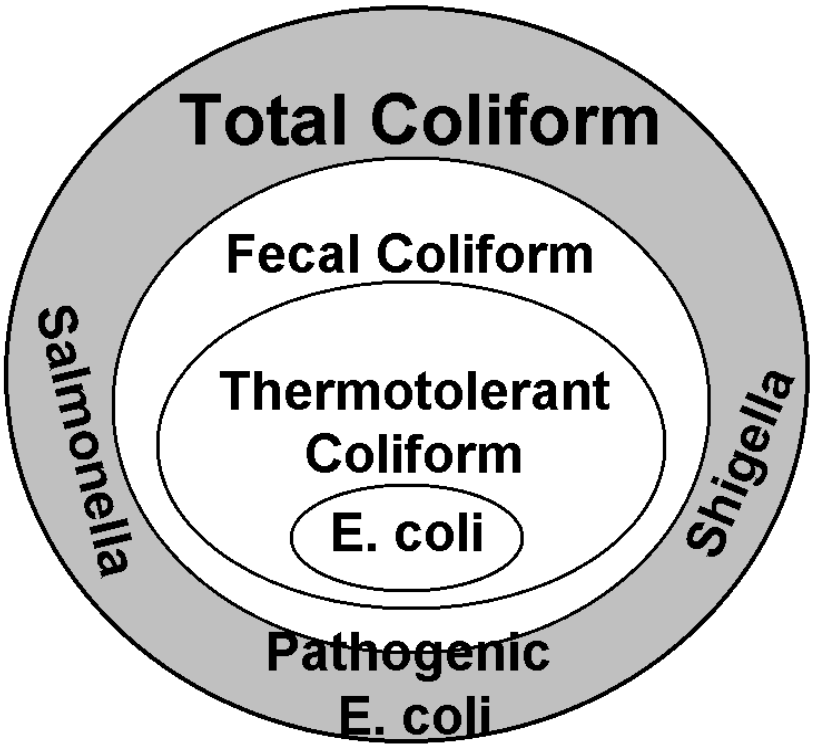
Protozoan pathogens are larger than bacteria and viruses, but still microscopic. They invade and inhabit the gastrointestinal tract. Some parasites enter the environment in a dormant form, with a protective cell wall called a "***cyst***." The cyst can survive in the environment for long periods of time and be extremely resistant to conventional disinfectants such as chlorine. Effective filtration treatment is therefore critical to removing these organisms from water sources.

Giardiasis
Giardiasis is a commonly reported protozoan-caused disease. It has also been referred to as "***backpacker's disease***" and "***beaver fever***" because of the many cases reported among hikers and others who consume untreated surface water. Symptoms include chronic diarrhea, abdominal cramps, bloating, frequent loose and pale greasy stools, fatigue and weight loss. The incubation period is 5-25 days or longer, with an average of 7-10 days. Many infections are asymptomatic (no symptoms). Giardiasis occurs worldwide. Waterborne outbreaks in the United States occur most often in communities receiving their drinking water from streams or rivers without adequate disinfection or a filtration system. The organism, ***Giardia lamblia,*** has been responsible for more community-wide outbreaks of disease in the U.S. than any other pathogen. Drugs are available for treatment but are not 100% effective.

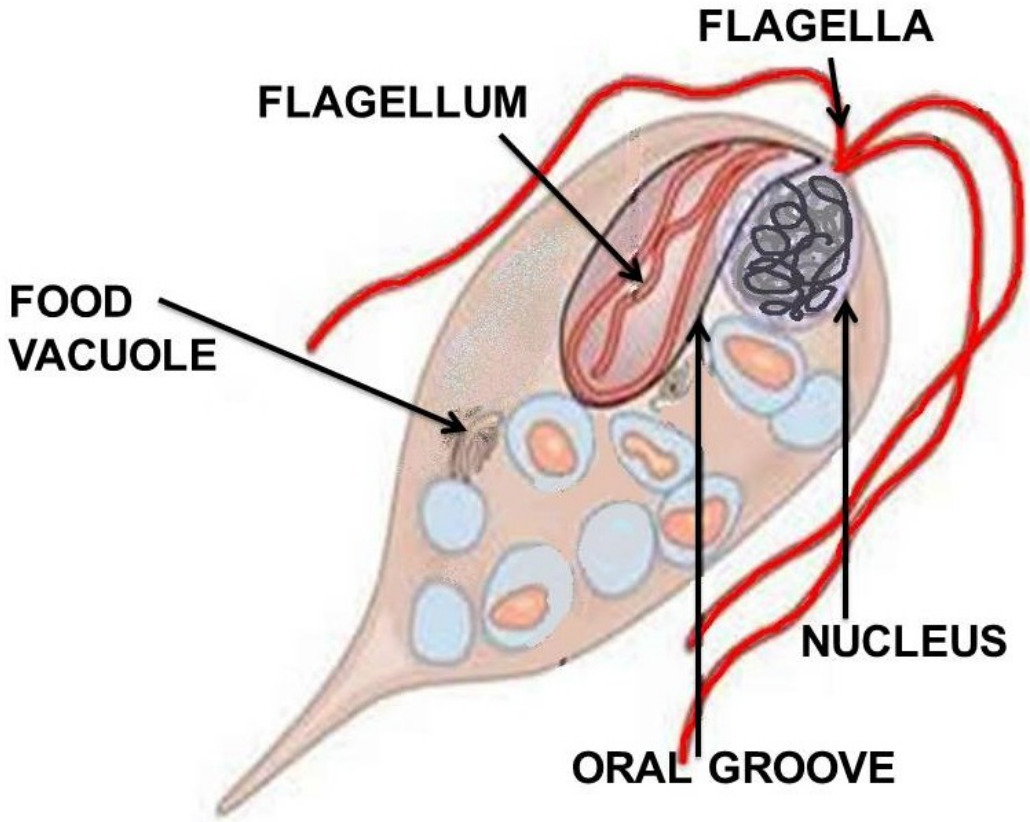
Cryptosporidiosis
Cryptosporidiosis is an example of a protozoan disease that is common worldwide, but was only recently recognized as causing human disease. The major symptom in humans is diarrhea, which may be profuse and watery. The diarrhea is associated with cramping abdominal pain. General malaise, fever, anorexia, nausea, and vomiting occur less often. Symptoms usually come and go, and end in fewer than 30 days in most cases. The incubation period is 1-12 days, with an average of about seven days. ***Cryptosporidium*** organisms have been identified in human fecal specimens from more than 50 countries on six continents. The mode of transmission is fecal-oral, either by person-to-person or animal-to-person. There is no specific treatment for ***Cryptosporidium*** infections.

All of these diseases, with the exception of hepatitis A, have one symptom in common: diarrhea. They also have the same mode of transmission, fecal-oral, whether through person-to-person or animal-to-person contact, and the same routes of transmission, being either foodborne or waterborne. Although most pathogens cause mild, self-limiting disease, on occasion, they can cause serious, even life threatening illness. Particularly vulnerable are persons with weak immune systems such as those with HIV infections or cancer. By understanding the nature of waterborne diseases, the importance of properly constructed, operated and maintained public water systems becomes obvious. While water treatment cannot achieve sterile water (no microorganisms), the goal of treatment must clearly be to produce drinking water that is as pathogen-free as possible at all times. For those who operate water systems with inadequate source protection or treatment facilities, the potential risk of a waterborne disease outbreak is real. For those operating systems that currently provide adequate source protection and treatment, operating and maintaining the system at a high level on a continuing basis is critical to prevent disease.




Total Coliform
Fecal Coliform
Thermotolerant Coliform
E. coli
Salmonella
Shigella
Pathogenic E. coli

Giardia Lamblia



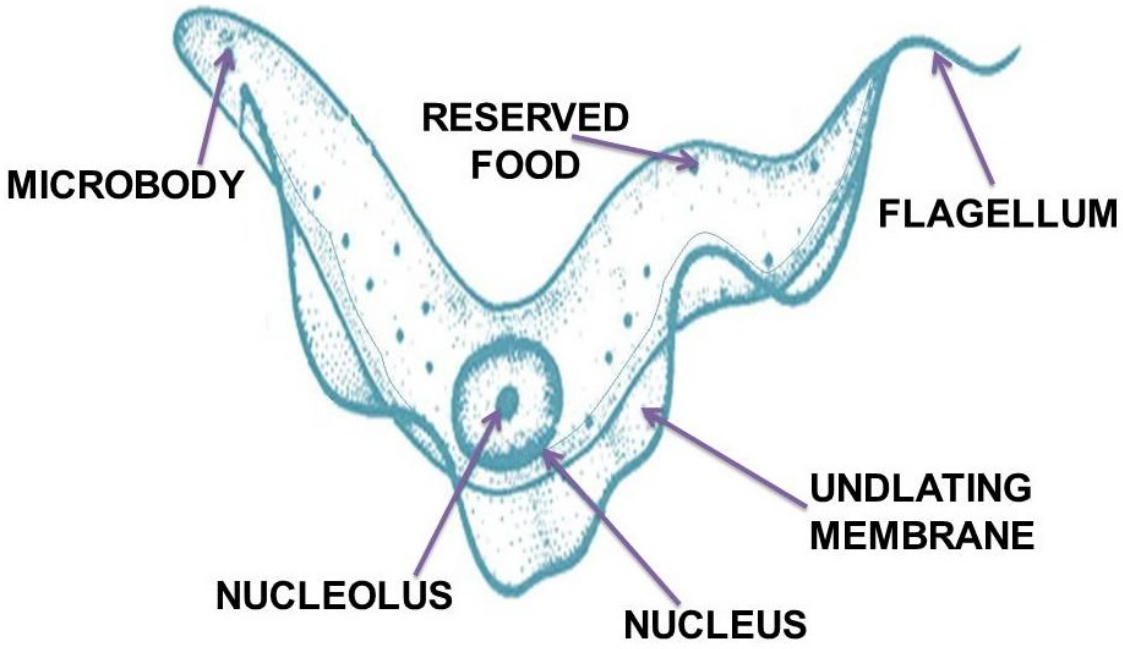
Giardia lamblia (synonymous with Lamblia intestinalis and Giardia duodenalis) is a flagellated protozoan parasite that colonizes and reproduces in the small intestine, causing giardiasis. The giardia parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastro-intestinal tract, but remains confined to the lumen of the small intestine. Giardia trophozoites absorb their nutrients from the lumen of the small intestine, and are anaerobes.

Giardia infection can occur through ingestion of dormant cysts in contaminated water, or by the fecal-oral route (through poor hygiene practices). The Giardia cyst can survive for weeks to months in cold water and therefore can be present in contaminated wells and water systems, and even clean-looking mountain streams, as well as city reservoirs, as the Giardia cysts are resistant to conventional water treatment methods, such as chlorination and ozonolysis. Zoonotic transmission is also possible, and therefore Giardia infection is a concern for people camping in the wilderness or swimming in contaminated streams or lakes, especially the artificial lakes formed by beaver dams (hence the popular name for giardiasis, "Beaver Fever"). As well as water-borne sources, fecal-oral transmission can also occur, for example in day care centers, where children may have poorer hygiene practices.

Those who work with children are also at risk of being infected, as are family members of infected individuals. Not all Giardia infections are symptomatic, so some people can unknowingly serve as carriers of the parasite.

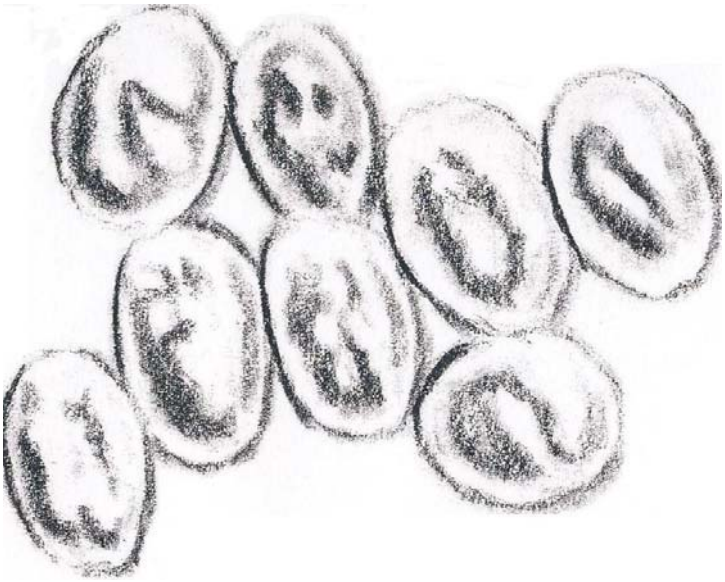
The life cycle begins with a non-infective cyst being excreted with feces of an infected individual. Once out in the environment, the cyst becomes infective. A distinguishing characteristic of the cyst is 4 nuclei and a retracted cytoplasm. Once ingested by a host, the trophozoite emerges to an active state of feeding and motility. After the feeding stage, the trophozoite undergoes asexual replication through longitudinal binary fission. The resulting trophozoites and cysts then pass through the digestive system in the feces. While the trophozoites may be found in the feces, only the cysts are capable of surviving outside of the host.

Distinguishing features of the trophozoites are large karyosomes and lack of peripheral chromatin, giving the two nuclei a halo appearance. Cysts are distinguished by a retracted cytoplasm. This protozoa lacks mitochondria, although the discovery of the presence of mitochodrial remnant organelles in one recent study "indicate that Giardia is not primitively amitochondrial and that it has retained a functional organelle derived from the original mitochondrial endosymbiont"



PROTOZOAN PARASITE

Cryptosporidium

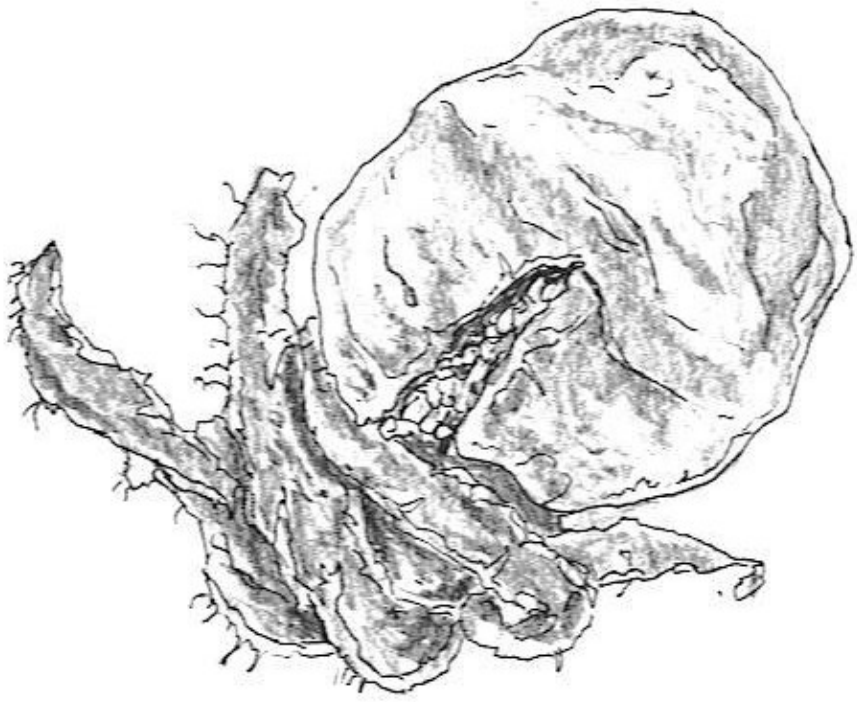


Cryptosporidium is a protozoan pathogen of the Phylum Apicomplexa and causes a diarrheal illness called cryptosporidiosis. Other apicomplexan pathogens include the malaria parasite Plasmodium, and Toxoplasma, the causative agent of toxoplasmosis. Unlike Plasmodium, which transmits via a mosquito vector, Cryptosporidium does not utilize an insect vector and is capable of completing its life cycle within a single host, resulting in cyst stages which are excreted in feces and are capable of transmission to a new host.

A number of species of Cryptosporidium infect mammals. In humans, the main causes of disease are C. parvum and C. hominis (previously C. parvum genotype 1). C. canis, C. felis, C. meleagridis, and C. muris can also cause disease in humans. In recent years, cryptosporidiosis has plagued many commercial Leopard gecko breeders. Several species of the Cryptosporidium family (C. serpentes and others) are involved, and outside of geckos it has been found in monitor lizards, iguanas, tortoises as well as several snake species.

Cryptosporidiosis is typically an acute short-term infection but can become severe and non-resolving in children and immunocompromised individuals. The parasite is transmitted by environmentally hardy cysts (oocysts) that, once ingested, excyst in the small intestine and result in an infection of intestinal epithelial tissue.

The genome of Cryptosporidium parvum was sequenced in 2004 and was found to be unusual amongst Eukaryotes in that the mitochondria seem not to contain DNA. A closely-related species, C. hominis, also has its genome sequence available. CryptoDB.org is a NIH-funded database that provides access to the Cryptosporidium genomics data sets.



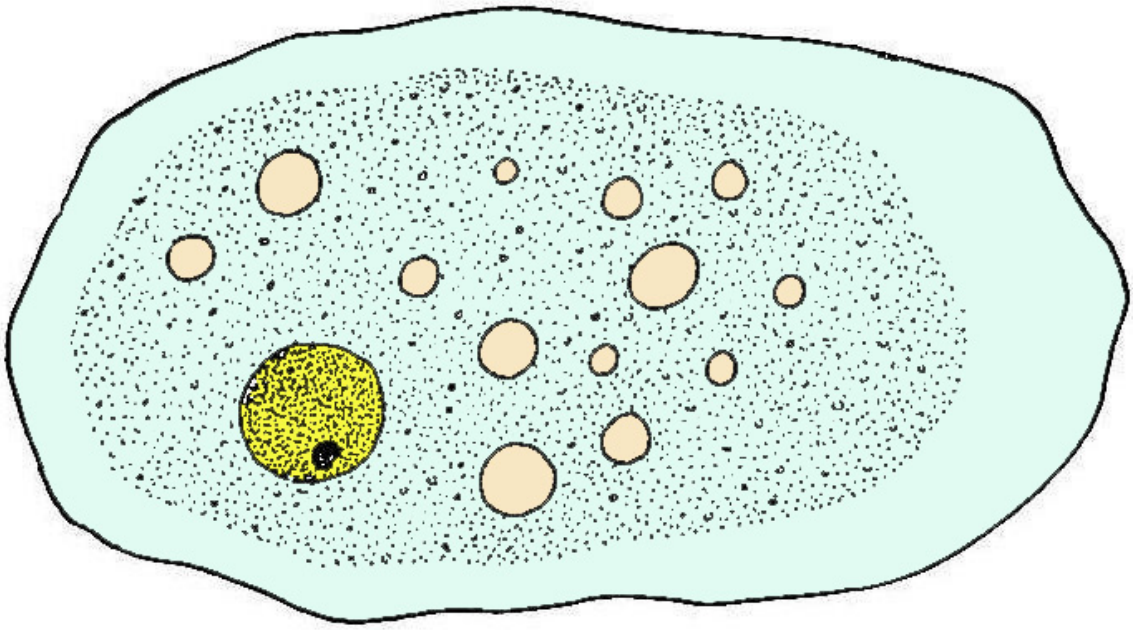
CRYPTO-PARVUM

When *C. parvum* was first identified as a human pathogen, diagnosis was made by a biopsy of intestinal tissue (Keusch, *et al*., 1995). However, this method of testing can give false negatives due the "patchy" nature of the intestinal parasitic infection (Flanigan and Soave, 1993). Staining methods were then developed to detect and identify the oocysts directly from stool samples. The modified acid-fast stain is traditionally used to most reliably and specifically detect the presence of cryptosporidial oocysts.

There have been six major outbreaks of cryptosporidiosis in the United States as a result of contamination of drinking water (Juranek, 1995). One major outbreak in Milwaukee in 1993 affected over 400,000 persons. Outbreaks such as these usually result from drinking water taken from surface water sources such as lakes and rivers (Juranek, 1995). Swimming pools and water park wave pools have also been associated with outbreaks of cryptosporidiosis. Also, untreated groundwater or well water public drinking water supplies can be sources of contamination.

The highly environmentally resistant cyst of *C. parvum* allows the pathogen to survive various drinking water filtrations and chemical treatments such as chlorination. Although municipal drinking water utilities may meet federal standards for safety and quality of drinking water, complete protection from cryptosporidial infection is not guaranteed. In fact, *all* waterborne outbreaks of cryptosporidiosis have occurred in communities where the local utilities met all state and federal drinking water standards (Juranek, 1995).

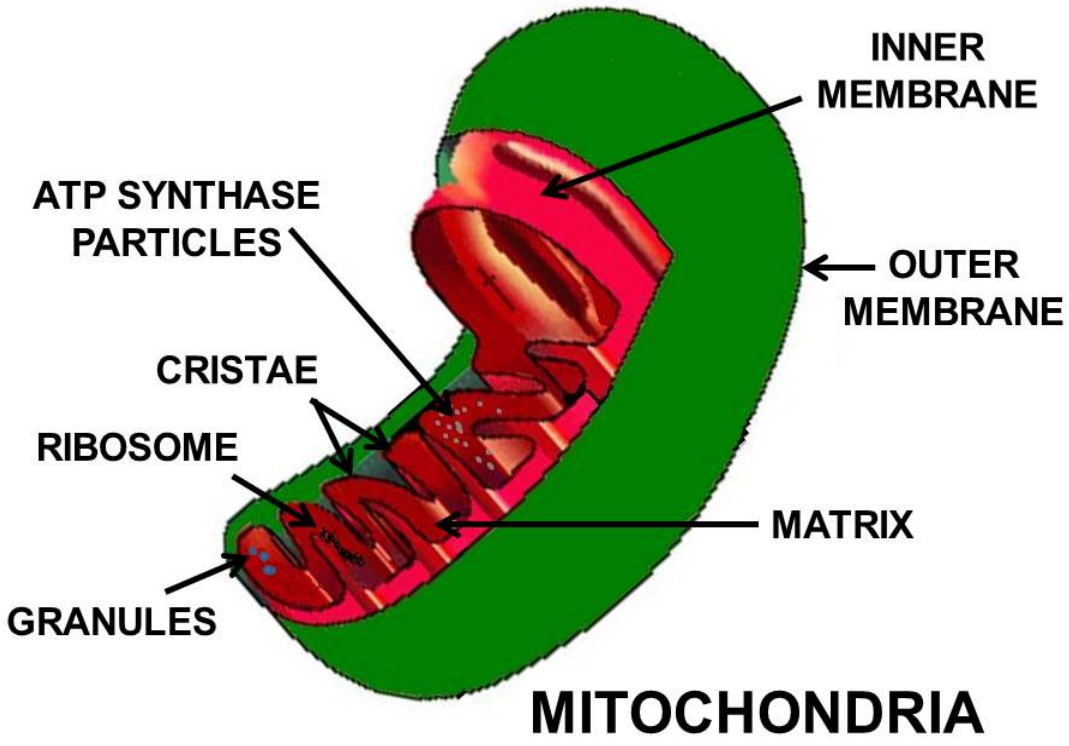
Entamoeba histolytica



Entamoeba histolytica, another water-borne pathogen, can cause diarrhea or a more serious invasive liver abscess. When in contact with human cells, these amoebae are cytotoxic. There is a rapid influx of calcium into the contacted cell, it quickly stops all membrane movement save for some surface blebbing. Internal organization is disrupted, organelles lyse, and the cell dies. The ameba may eat the dead cell or just absorb nutrients released from the cell.

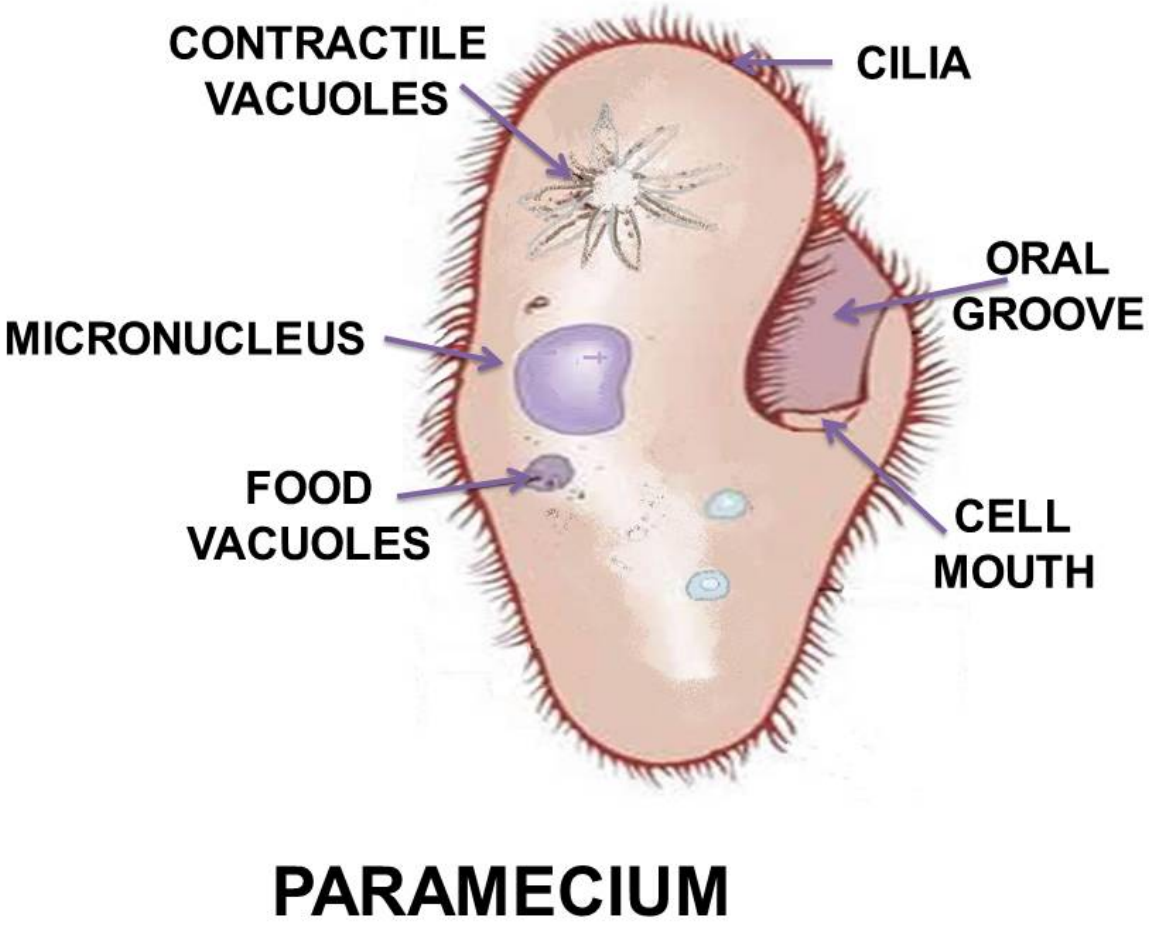
On average, about one in 10 people who are infected with *E. histolytica* becomes sick from the infection. The symptoms often are quite mild and can include loose stools, stomach pain, and stomach cramping. Amebic dysentery is a severe form of amebiasis associated with stomach pain, bloody stools, and fever. Rarely, *E. histolytica* invades the liver and forms an abscess. Even less commonly, it spreads to other parts of the body, such as the lungs or brain.

Scientific classification
Domain: Eukaryota
Phylum: Amoebozoa
Class: Archamoebae
Genus: Entamoeba
Species: E. histolytica



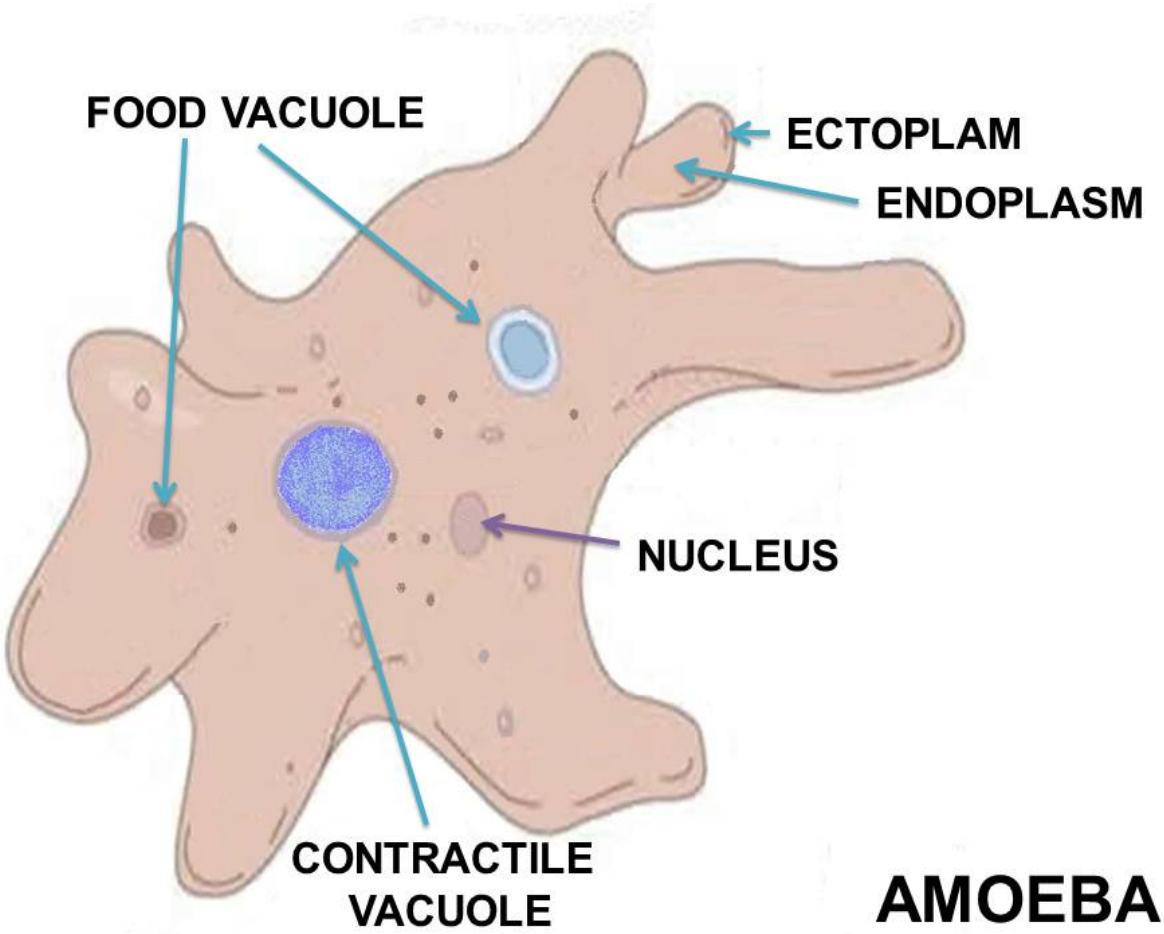
Mitochondria

The bacterial cell is surrounded by a lipid membrane, or cell membrane, which encloses the contents of the cell and acts as a barrier to hold nutrients, proteins and other essential components of the cytoplasm within the cell. As they are prokaryotes, bacteria do not tend to have membrane-bound organelles in their cytoplasm and thus contain few large intracellular structures. They consequently lack a nucleus, mitochondria, chloroplasts and the other organelles present in eukaryotic cells, such as the Golgi apparatus and endoplasmic reticulum.



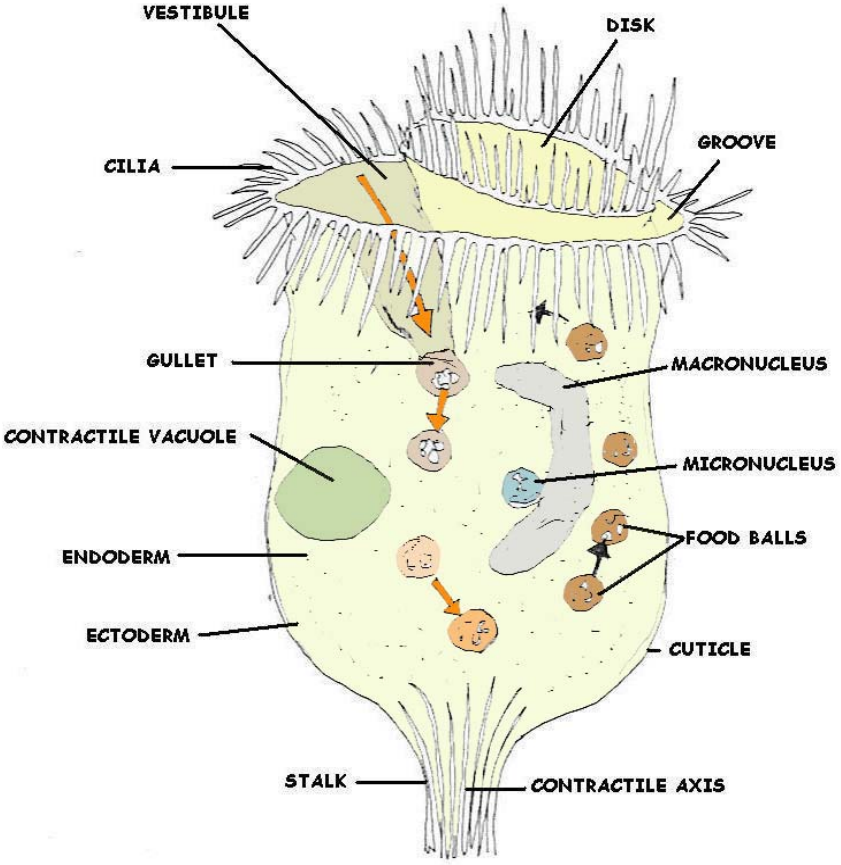
Paramecia

Paramecia are a group of unicellular ciliate protozoa formerly known as slipper animalcules from their slipper shape. They are commonly studied as a representative of the ciliate group. Simple cilia cover the body which allows the cell to move with a synchronous motion (like a caterpilla). There is also a deep oral groove containing inconspicuous compound oral cilia (as found in other peniculids) that is used to draw food inside. They generally feed upon bacteria and other small cells. Osmoregulation is carried out by a pair of contractile vacuoles, which actively expel water absorbed by osmosis from their surroundings. Paramecia are widespread in freshwater environments, and are especially common in scums. Paramecia are attracted by acidic conditions. Certain single-celled eukaryotes, such as Paramecium, are examples for exceptions to the universality of the genetic code (translation systems where a few codons differ from the standard ones).



Amoeba

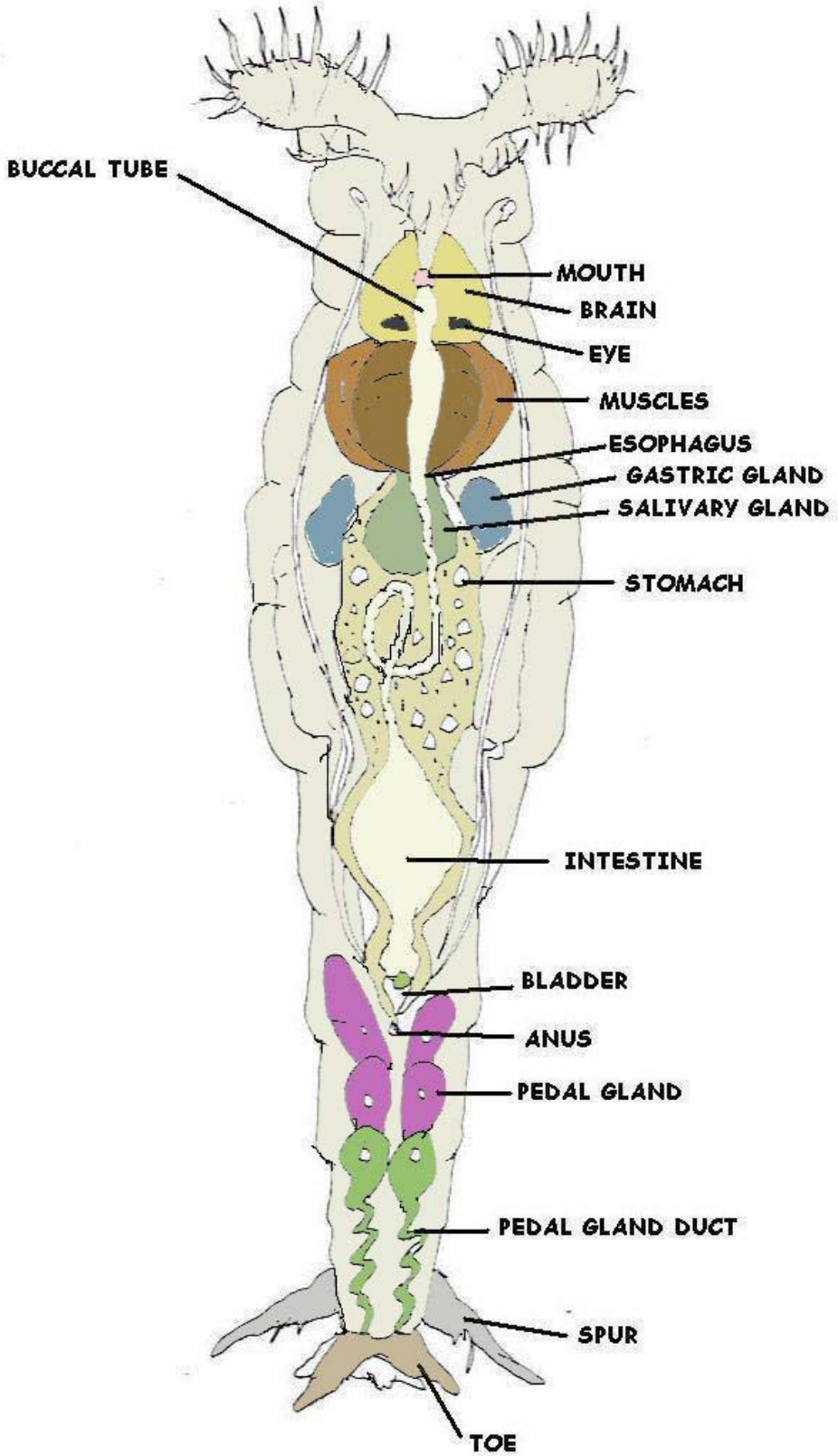
Amoeba (sometimes amœba or ameba, plural amoebae) is a genus of protozoa that moves by means of pseudopods, and is well-known as a representative unicellular organism. The word amoeba or ameba is variously used to refer to it and its close relatives, now grouped as the Amoebozoa, or to all protozoa that move using pseudopods, otherwise termed amoeboids.



Vorticella

Vorticella is a genus of protozoa, with over 100 known species. They are stalked inverted bell-shaped ciliates, placed among the peritrichs. Each cell has a separate stalk anchored onto the substrate, which contains a contracile fibril called a myoneme. When stimulated this shortens, causing the stalk to coil like a spring. Reproduction is by budding, where the cell undergoes longitudinal fission and only one daughter keeps the stalk. Vorticella mainly lives in freshwater ponds and streams - generally anywhere protists are plentiful. Other genera such as Carchesium resemble Vorticella but are branched or colonial.

Domain: Eukaryota
Phylum: Ciliophora
Class: Oligohymenophorea
Subclass: Peritrichia
Order: Sessilida
Family: Vorticellidae
Genus: Vorticella


BUCCAL TUBE
MOUTH
BRAIN
EYE
MUSCLES
ESOPHAGUS
GASTRIC GLAND
SALIVARY GLAND
STOMACH
INTESTINE
BLADDER
ANUS
PEDAL GLAND
PEDAL GLAND DUCT
SPUR
TOE

ROTIFER

Rotifer

The rotifers make up a phylum of microscopic and near-microscopic pseudocoelomate animals. They were first described by John Harris in 1696 (Hudson and Gosse, 1886). Leeuwenhoek is mistakenly given credit for being the first to describe rotifers but Harris had produced sketches in 1703. Most rotifers are around 0.1-0.5 mm long, and are common in freshwater throughout the world with a few saltwater species. Rotifers may be free swimming and truly planktonic, others move by inch worming along the substrate, whilst some are sessile, living inside tubes or gelatinous holdfasts. About 25 species are colonial (e.g. Sinantherina semibullata), either sessile or planktonic.

Rotifers get their name (derived from Greek and meaning "wheel-bearer"; they have also been called wheel animalcules) from the corona, which is composed of several ciliated tufts around the mouth that in motion resemble a wheel. These create a current that sweeps food into the mouth, where it is chewed up by a characteristic pharynx (called the mastax) containing a tiny, calcified, jaw-like structure called the trophi. The cilia also pull the animal, when unattached, through the water. Most free-living forms have pairs of posterior toes to anchor themselves while feeding. Rotifers have bilateral symmetry and a variety of different shapes. There is a well-developed cuticle which may be thick and rigid, giving the animal a box-like shape, or flexible, giving the animal a worm-like shape; such rotifers are respectively called loricate and illoricate.

Like many other microscopic animals, adult rotifers frequently exhibit eutely - they have a fixed number of cells within a species, usually on the order of one thousand. Males in the class Monogononta may be either present or absent depending on the species and environmental conditions. In the absence of males, reproduction is by parthenogenesis and results in clonal offspring that are genetically identical to the parent. Individuals of some species form two distinct types of parthenogenetic eggs; one type develops into a normal parthenogenetic female, while the other occurs in response to a changed environment and develops into a degenerate male that lacks a digestive system, but does have a complete male reproductive system that is used to inseminate females thereby producing fertilized 'resting eggs'. Resting eggs develop into zygotes that are able to survive extreme environmental conditions such as may occur during winter or when the pond dries up. These eggs resume development and produce a new female generation when conditions improve again. The life span of monogonont females varies from a couple of days to about three weeks.

Bdelloid rotifers are unable to produce resting eggs, but many can survive prolonged periods of adverse conditions after desiccation. This facility is termed anhydrobiosis, and organisms with these capabilities are termed anhydrobionts. Under drought conditions, bdelloid rotifers contract into an inert form and lose almost all body water; when rehydrated, however, they resume activity within a few hours. Bdelloids can survive the dry state for prolonged periods, with the longest well-documented dormancy being nine years. While in other anhydrobionts, such as the brine shrimp, this desiccation tolerance is thought to be linked to the production of trehalose, a non-reducing disaccharide (sugar), bdelloids apparently lack the ability to synthesize trehalose. Bdelloid rotifer genomes contain two or more divergent copies of each gene. Four copies of hsp82 are, for example, found. Each is different and found on a different chromosome, excluding the possibility of homozygous sexual reproduction.

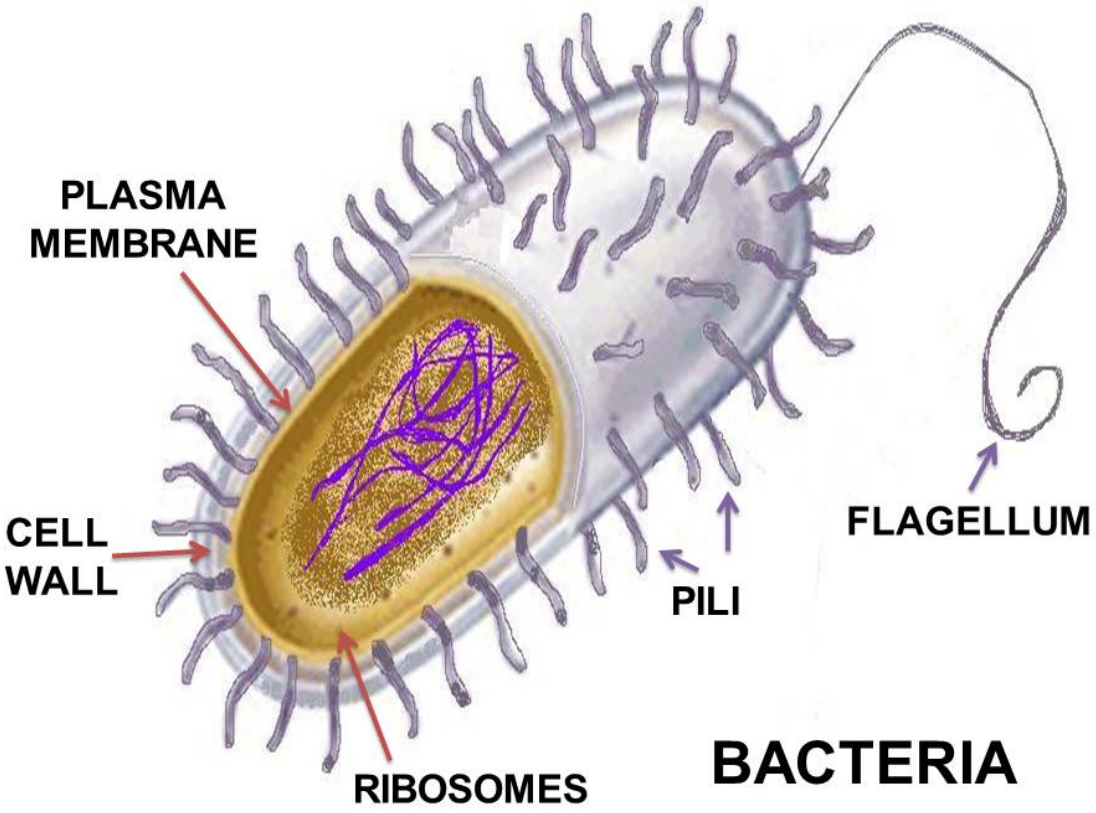
Waterborne Diseases

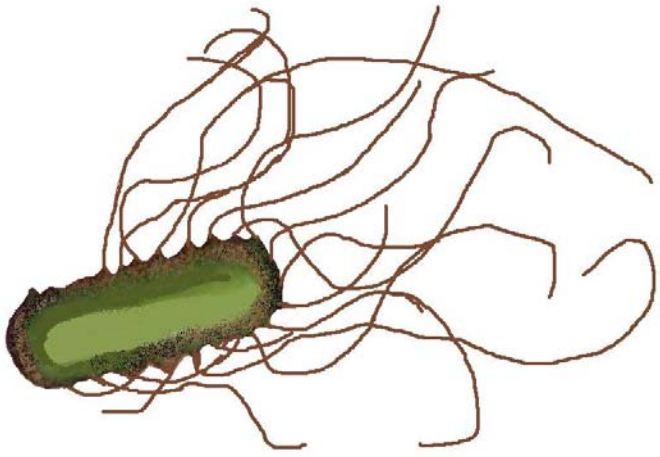
Name	Causative organism	Source of organism	Disease
Viral gastroenteritis	**Rotavirus** (mostly in young children)	Human feces	Diarrhea or vomiting
Norwalk Agent	**Noroviruses** (genus *Norovirus*, family *Caliciviridae*) [*1]	Human feces; also, shellfish; lives in polluted waters	Diarrhea and vomiting
Salmonellosis	***Salmonella*** (bacterium)	Animal or human feces	Diarrhea or vomiting
Gastroenteritis Escherichia *coli*	-- ***E. coli O1* 57:H7** (bacterium): Other *E. coli* organisms:	Human feces	Symptoms vary with type caused
Typhoid	***Salmonella typhi*** (bacterium)	Human feces, urine	Inflamed intestine, enlarged spleen, high temperature-sometimes fatal
Shigellosis	***Shigella*** (bacterium)	Human feces	Diarrhea
Cholera	***Vibrio choleras*** (bacterium)	Human feces; also, shellfish; lives in many coastal waters	Vomiting, severe diarrhea, rapid dehydration, mineral loss-high mortality
Hepatitis A	**Hepatitis A** virus	Human feces; shellfish grown in polluted waters	Yellowed skin, enlarged liver, fever, vomiting, weight loss, abdominal pain-low mortality, lasts up to four months
Amebiasis	***Entamoeba histolytica*** (protozoan)	Human feces	Mild diarrhea, dysentery, extra intestinal infection
Giardiasis	***Giardia lamblia*** (protozoan)	Animal or human feces	Diarrhea, cramps, nausea, and general weakness — lasts one week to months
Cryptosporidiosis	***Cryptosporidium parvum***	Animal or human feces	Diarrhea, stomach pain — lasts (protozoan) days to weeks

Notes:

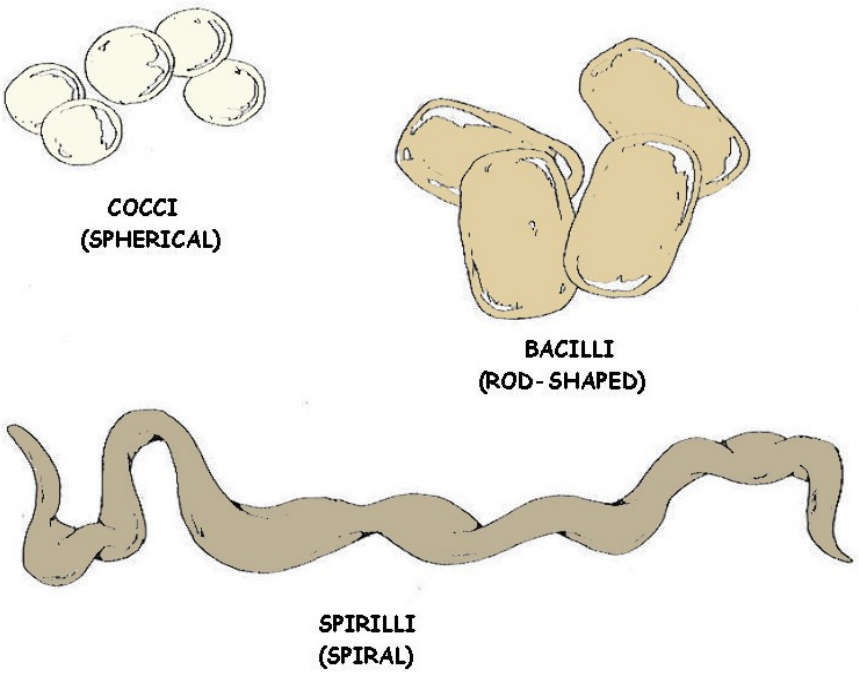
*1 http://www.cdc.gov/ncidod/dvrd/revb/gastro/norovirus.htm
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5009a1.htm

Bacteria Section



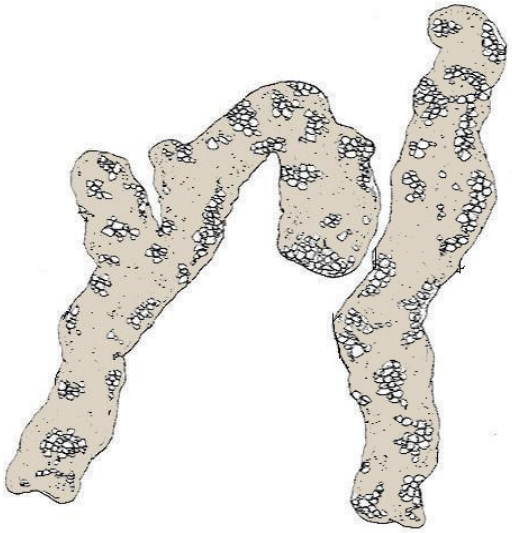


Peritrichous Bacteria



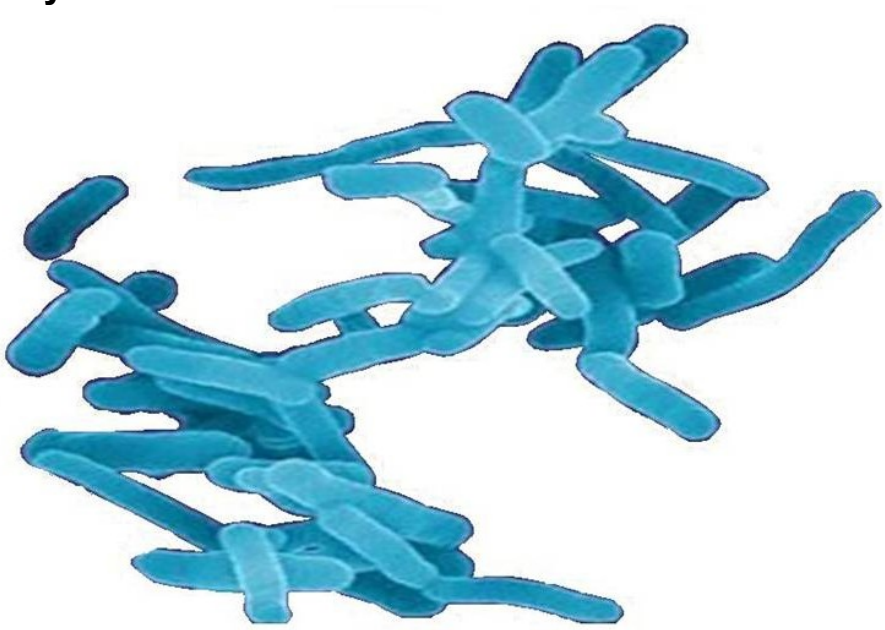
BACTERIA TYPES

Microbiologists broadly classify bacteria according to their shape: spherical, rod-shaped, and spiral-shaped. Pleomorphic bacteria can assume a variety of shapes. Bacteria may be further classified according to whether they require oxygen (aerobic or anaerobic) and how they react to a test with Gram's stain. Bacteria in which alcohol washes away Gram's stain are called gram-negative, while bacteria in which alcohol causes the bacteria's walls to absorb the stain are called gram-positive.



COLORLESS FILAMENTOUS
SULFUR BACTERIA

Shigella dysenteriae



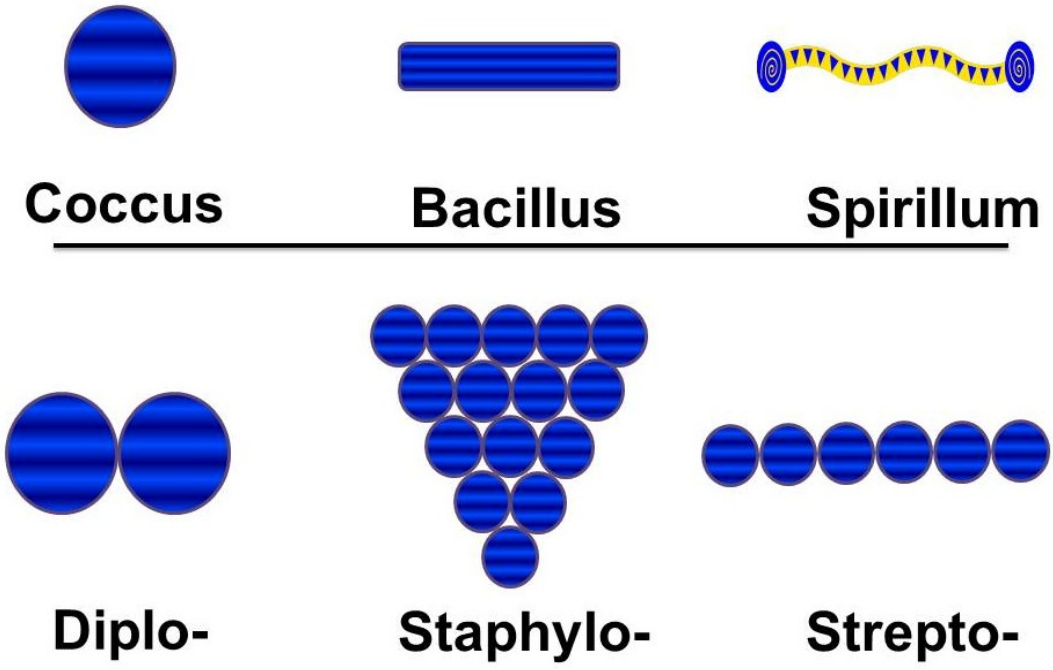
SHIGELLA DYSENTERIAE

Shigella dysenteriae is a species of the rod-shaped bacterial genus Shigella. Shigella can cause shigellosis (bacillary dysentery). Shigellae are Gram-negative, non-spore-forming, facultatively anaerobic, non-motile bacteria.

S. dysenteriae, spread by contaminated water and food, causes the most severe dysentery because of its potent and deadly Shiga toxin, but other species may also be dysentery agents. Shigella infection is typically via ingestion (fecal–oral contamination); depending on age and condition of the host as few as ten bacterial cells can be enough to cause an infection. Shigella causes dysentery that result in the destruction of the epithelial cells of the intestinal mucosa in the cecum and rectum. Some strains produce enterotoxin and Shiga toxin, similar to the verotoxin of E. coli O157:H7. Both Shiga toxin and verotoxin are associated with causing hemolytic uremic syndrome.

Shigella invades the host through epithelial cells of the large intestine. Using a Type III secretion system acting as a biological syringe, the bacterium injects IpaD protein into cell, triggering bacterial invasion and the subsequent lysis of vacuolar membranes using IpaB and IpaC proteins. It utilizes a mechanism for its motility by which its IcsA protein triggers actin polymerization in the host cell (via N-WASP recruitment of Arp2/3 complexes) in a "rocket" propulsion fashion for cell-to-cell spread.

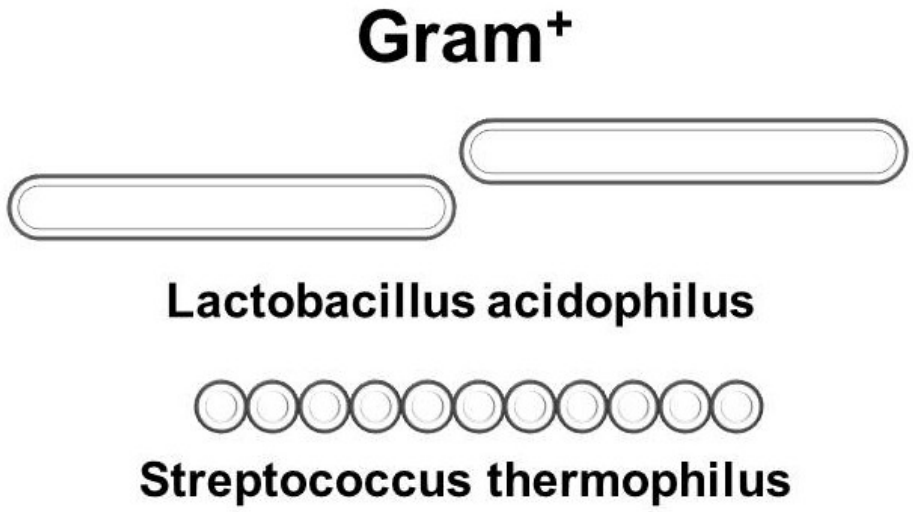
The most common symptoms are diarrhea, fever, nausea, vomiting, stomach cramps, and straining to have a bowel movement. The stool may contain blood, mucus, or pus (e.g. dysentery). In rare cases, young children may have seizures. Symptoms can take as long as a week to show up, but most often begin two to four days after ingestion. Symptoms usually last for several days, but can last for weeks. Shigella is implicated as one of the pathogenic causes of reactive arthritis worldwide.

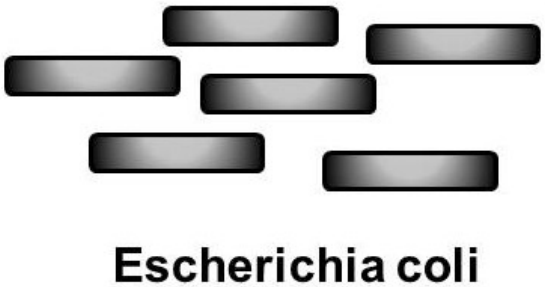


Bacteria Types

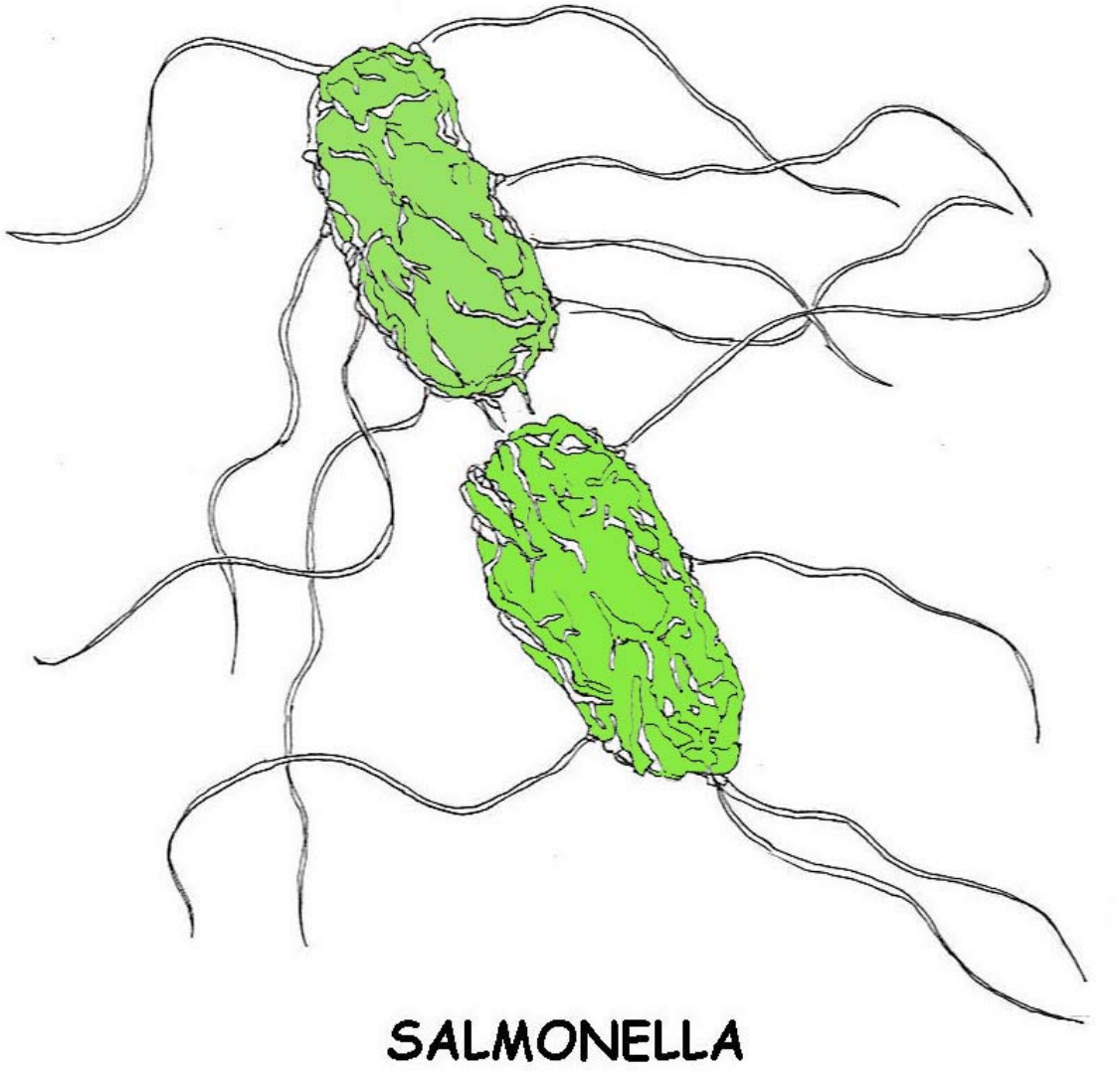
Type	Characteristics
Acetic acid	Rod-shaped, gram-negative, aerobic; highly tolerant of acidic conditions; generate organic acids
Actinomycete	Rod-shaped or filamentous, gram-positive, aerobic; common in soils; essential to growth of many plants; source of much of original antibiotic production in pharmaceutical industry
Coccoid	Spherical, sometimes in clusters or strings, gram-positive, aerobic and anaerobic; resistant to drying and high-salt conditions; *Staphylococcus* species common on human skin, certain strains associated with toxic shock syndrome
Coryneform	Rod-shaped, form club or V shapes, gram-positive, aerobic; found in wide variety of habitats, particularly soils; highly resistant to drying; include *Arthrobacter,* among most common forms of life on earth
Endospore-forming	Usually rod-shaped, can be gram-positive or gram-negative; have highly adaptable, heat-resistant spores that can go dormant for long periods, possibly thousands of years; include *Clostridium* (anaerobic) and *Bacillus* (aerobic)
Enteric	Rod-shaped, gram-negative, aerobic but can live in certain anaerobic conditions; produce nitrite from nitrate, acids from glucose; include *Escherichia coli, Salmonella* (over 1000 types), and *Shigella*
Gliding	Rod-shaped, gram-negative, mostly aerobic; glide on secreted slimy substances; form colonies, frequently with complex fruiting structures
Lactic acid	Gram-positive, anaerobic; produce lactic acid through fermentation; include *Lactobacillus*, essential in dairy product formation, and *Streptococcus*, common in humans
Mycobacterium	Pleomorphic, spherical or rod-shaped, frequently branching, no gram stain, aerobic; commonly form yellow pigments; include *Mycobacterium tuberculosis,* cause of tuberculosis
Mycoplasma	Spherical, commonly forming branching chains, no gram stain, aerobic but can live in certain anaerobic conditions; without cell walls yet structurally resistant to lysis; among smallest of bacteria; named for superficial resemblance to fungal hyphae (*myco-* means 'fungus')
Nitrogen-fixing	Rod-shaped, gram-negative, aerobic; convert atmospheric nitrogen gas to ammonium in soil; include *Azotobacter,* a common genus
Propionic acid	Rod-shaped, pleomorphic, gram-positive, anaerobic; ferment lactic acid; fermentation produces holes in Swiss cheese from the production of carbon dioxide
Pseudomonad	Rod-shaped (straight or curved) with polar flagella, gram-negative, aerobic; can use up to 100 different compounds for carbon and energy
Rickettsia	Spherical or rod-shaped, gram-negative, aerobic; cause Rocky Mountain spotted fever and typhus; closely related to *Agrobacterium,* a common gall-causing plant bacterium
Sheathed	Filamentous, gram-negative, aerobic; 'swarmer' (colonizing) cells form and break out of a sheath; sometimes coated with metals from environment

Spirillum	Spiral-shaped, gram-negative, aerobic; include *Bdellovibrio,* predatory on other bacteria
Spirochete	Spiral-shaped, gram-negative, mostly anaerobic; common in moist environments, from mammalian gums to coastal mudflats; complex internal structures convey rapid movement; include *Treponemapallidum,* cause of syphilis
Sulfate- and Sulfur-reducing	Commonly rod-shaped, mostly gram-negative, anaerobic; include *Desulfovibrio,* ecologically important in marshes
Sulfur- and iron-oxidizing	Commonly rod-shaped, frequently with polar flagella, gram-negative, mostly anaerobic; most live in neutral (nonacidic) environment
Vibrio	Rod- or comma-shaped, gram-negative, aerobic; commonly with a single flagellum; include *Vibrio cholerae,* cause of cholera, and luminescent forms symbiotic with deep-water fishes and squids





Salmonella



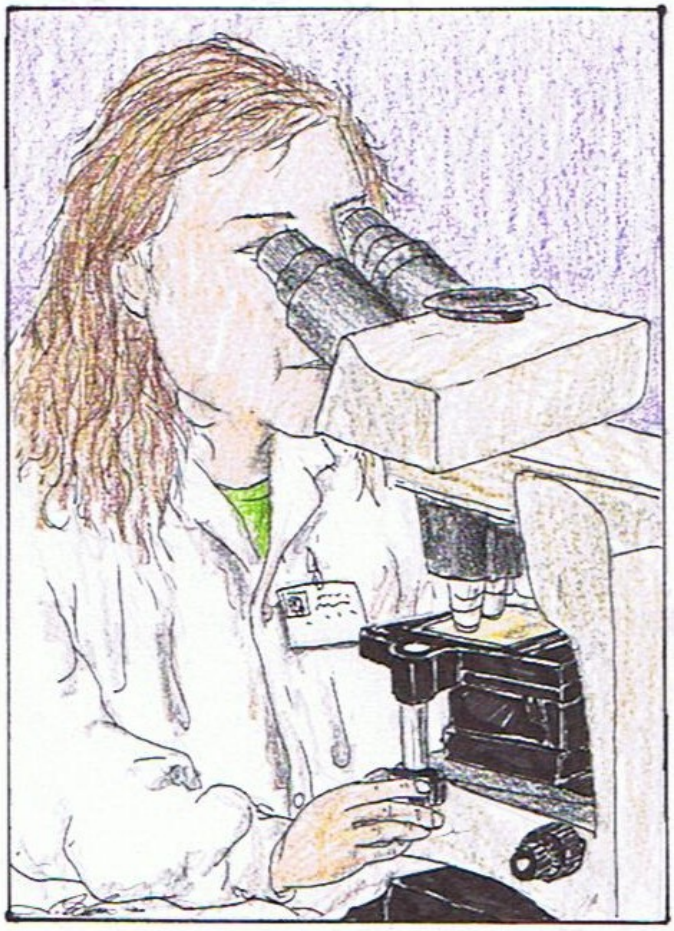
Salmonella is a Gram-negative bacterium. It is found in many turtles and other reptiles. In clinical laboratories, it is usually isolated on MacConkey agar, XLD agar, XLT agar, DCA agar, or Önöz agar. Because they cause intestinal infections and are greatly outnumbered by the bacteria normally found in the healthy bowel, primary isolation requires the use of a selective medium, so use of a relatively non-selective medium such as CLED agar is not often practiced. Numbers of salmonella may be so low in clinical samples that stools are routinely also subjected to "enrichment culture", where a small volume of stool is incubated in a selective broth medium, such as selenite broth or Rappaport Vassiliadis soya peptone broth, overnight. These media are inhibitory to the growth of the microbes normally found in the healthy human bowel, while allowing salmonellae to become enriched in numbers. Salmonellae may then be recovered by inoculating the enrichment broth on one or more of the primary selective media. On blood agar, they form moist colonies about 2 to 3 mm in diameter.

When the cells are grown for a prolonged time at a range of 25—28°C, some strains produce a biofilm, which is a matrix of complex carbohydrates, cellulose and proteins. The ability to produce biofilm (a.k.a. "rugose", "lacy", or "wrinkled") can be an indicator of dimorphism, which is the ability of a single genome to produce multiple phenotypes in response to environmental conditions. Salmonellae usually do not ferment lactose; most of them produce hydrogen sulfide which, in media containing ferric ammonium citrate, reacts to form a black spot in the centre of the creamy colonies.

Classification

Salmonella taxonomy is complicated. As of December 7, 2005, there are two species within the genus: S. bongori (previously subspecies V) and S. enterica (formerly called S. choleraesuis), which is divided into six subspecies:

* I—enterica
* II—salamae
* IIIa—arizonae
* IIIb—diarizonae
* IV—houtenae
* V—obsolete (now designated S. bongori)
* VI—indica



There are also numerous (over 2500) serovars within both species, which are found in a disparate variety of environments and which are associated with many different diseases. The vast majority of human isolates (>99.5%) are subspecies S. enterica. For the sake of simplicity, the CDC recommends that Salmonella species be referred to only by their genus and serovar, e.g.

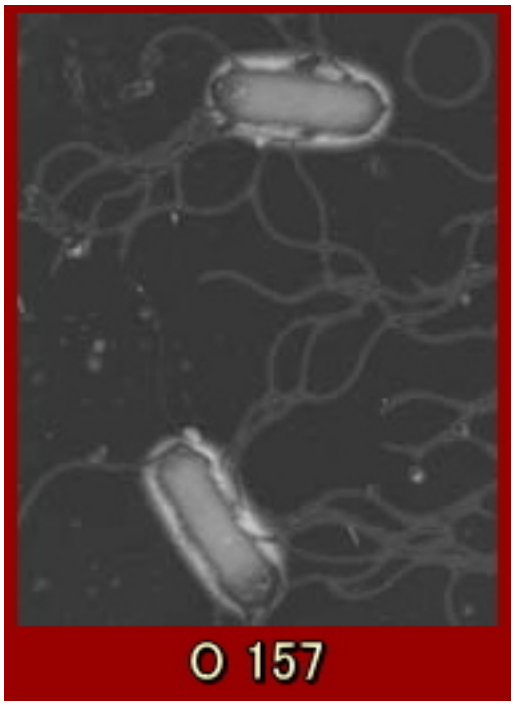
Salmonella Typhi instead of the more technically correct designation, Salmonella enterica subspecies enterica serovar Typhi.

Escherichia Coli Section

Fecal Coliform Bacteria
Fecal coliform bacteria are microscopic organisms that live in the intestines of warm-blooded animals. They also live in the waste material, or feces, excreted from the intestinal tract. When fecal coliform bacteria are present in high numbers in a water sample, it means that the water has received fecal matter from one source or another. Although not necessarily agents of disease, fecal coliform bacteria may indicate the presence of disease-carrying organisms, which live in the same environment as the fecal coliform bacteria.

Reasons for Natural Variation
Unlike the other conventional water quality parameters, fecal coliform bacteria are living organisms. They do not simply mix with the water and float straight downstream. Instead they multiply quickly when conditions are favorable for growth, or die in large numbers when conditions are not. Because bacterial concentrations are dependent on specific conditions for growth, and these conditions change quickly, fecal coliform bacteria counts are not easy to predict. For example, although winter rains may wash more fecal matter from urban areas into a stream, cool water temperatures may cause a major die-off. Exposure to sunlight (with its ultraviolet disinfection properties) may have the same effect, even in the warmer water of summertime.

Expected Impact of Pollution
The primary sources of fecal coliform bacteria to fresh water are wastewater treatment plant discharges, failing septic systems, and animal waste. Bacteria levels do not necessarily decrease as a watershed develops from rural to urban. Instead, urbanization usually generates new sources of bacteria. Farm animal manure and septic systems are replaced by domestic pets and leaking sanitary sewers. In fact, stormwater runoff in urbanized areas has been found to be surprisingly high in fecal coliform bacteria concentrations.



The presence of old, disintegrating storm and sanitary sewers, misplaced sewer pipes, and good breeding conditions are common explanations for the high levels measured.

Coliform Standards (in colonies/100ml)
Drinking water.....1FC
Total body contact (swimming).....200FC
Partial body contact (boating).....1000FC
Threatened sewage effluent .....not to exceed 200 FC

*Total coliform (TC) includes bacteria from cold-blooded animals and various soil organisms. According to recent literature, total coliform counts are normally about 10 times higher than fecal coliform (FC) counts.

Indicator Connection Varies
General coliforms, E. Coli, and Enterococcus bacteria are the "indicator" organisms generally measured to assess microbiological quality of water. However, these aren't generally what get people sick. Other bacteria, viruses, and parasites are what we are actually worried about.

Because it is so much more expensive and tedious to do so, actual pathogens are virtually never tested for. Over the course of a professional lifetime pouring over indicator tests, in a context where all standards are based on indicators, water workers tend to forget that the indicators are not the things we actually care about.

What are these indicators? More information in the Laboratory section.

- **General coliforms** indicate that the water has come in contact with plant or animal life. General coliforms are universally present, including in pristine spring water. They are of little concern at low levels, except to indicate the effectiveness of disinfection. Chlorinated water and water from perfectly sealed tube wells is the only water I've tested which had zero general coliforms. At very high levels they indicate there is what amounts to a lot of compost in the water, which could easily include pathogens (Ten thousand general coliform bacteria will get you a beach closure, compared to two or four hundred fecal coliforms, or fifty enterococcus).
- **Fecal coliforms**, particularly E. coli, indicate that there are mammal or bird feces in the water.
- **Enterococcus bacteria** also indicate that there are feces from warm blooded animals in the water. Enterococcus are a type of fecal streptococci. They are another valuable indicator for determining the amount of fecal contamination of water.
  According to studies conducted by the EPA, enterococci have a greater correlation with swimming-associated gastrointestinal illness in both marine and fresh waters than other bacterial indicator organisms, and are less likely to "die off" in saltwater.

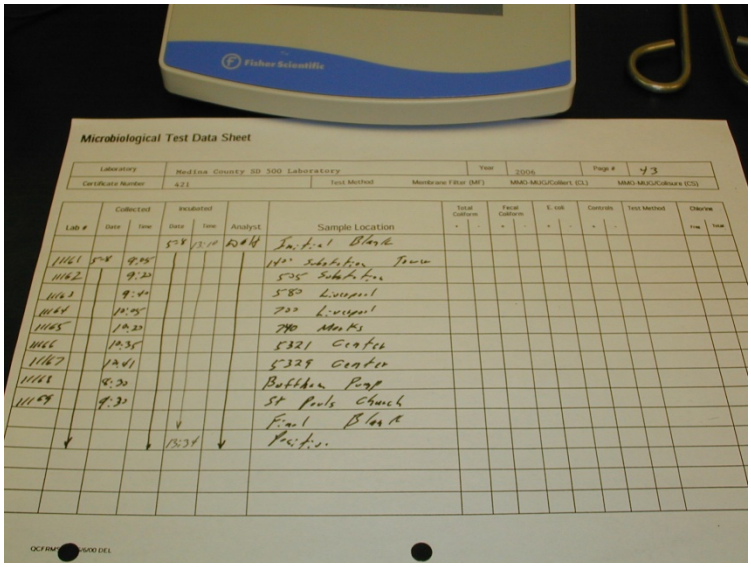
The more closely related the animal, the more likely pathogens excreted with their feces can infect us. Human feces are the biggest concern, because anything which infects one human could infect another. There isn't currently a quantitative method for measuring specifically human fecal bacteria (expensive genetic studies can give a presence/absence result). Ingesting a human stranger's feces via contaminated water supply is a classic means for infections to spread rapidly. The more pathogens an individual carries, the more hazardous their feces. Ingesting feces from someone who is not carrying any pathogens may gross you out, but it can't infect you. Infection rates are around 5% in the US, and approach 100% in areas with poor hygiene and contaminated water supplies. Keep in the back of your mind that **the ratio of indicators to actual pathogens is not fixed**. It will always be different, sometimes very different. Whenever you are trying to form a mental map of reality based on water tests, you should include in the application of your water intuition an adjustment factor for your best guess of the ratio between indicators and actual pathogens.

Membrane Filter Total Coliform Technique

The membrane filter total Coliform technique is used at Medina County for drinking water quality testing. The following is a summary of this test. A sampling procedure sheet is given to all sample takers by Medina County.

The samples are taken in sterile 100 mL containers. These containers, when used for chlorinated water samples, have a sodium thiosulfate pill or solution to dechlorinate the sample.

The sample is placed in cold storage after proper sample taking procedures are followed. (See sample procedures below)



The samples are taken to the laboratory with a chain of custody to assure no tampering of samples can occur.

These samples are logged in at the laboratory.

No longer than 30 hours can lapse between the time of sampling and time of test incubation. (8 hours for heterotrophic, nonpotable 6 hours, others not longer than 24 hours)

All equipment is sterilized by oven and autoclave.
Glassware in oven at 170°C ± 10°C with foil (or other suitable wrap) loosely fitting and secured immediately after sterilization.

Filtration units in autoclave at 121°C for 30 minutes.

Use sterile petri dishes, grid, and pads bought from a reliable company – certified, quality assured - test for satisfactory known positive amounts.

Incubators – 35°C ± .5°C (60% relative humidity)
M-endo medium is prepared and heated to near boiling removed from heat cooled to 45°C pH adjusted to 7.2 ± .2 and immediately dispensed 8ml to plates. Keep refrigerated and discard after 2 weeks.

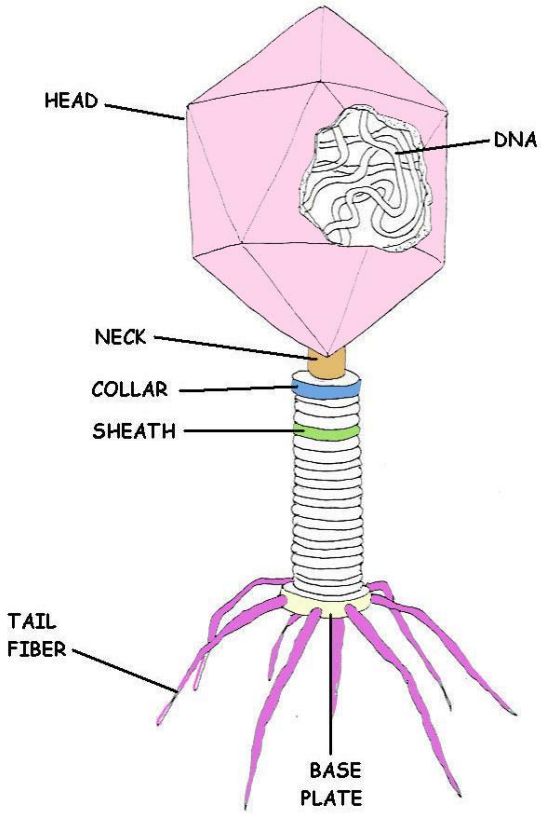
Plates can be stored in a dated box with expiration date and discarded if not used. No denatured alcohol should be used. Everclear or 95% proof alcohol or absolute methyl may be used for sterilizing forceps by flame.

Procedure:
Counters are alcohol wiped.
Bench sheets are filled out.
Samples are removed from refrigeration.
Sterile wrapped utensils are placed on counters.
Filtration units are placed onto sterile membrane filters by aseptic technique using sterile forceps.
Sterile petri dishes are labeled.
The samples closures are clipped.
The sample is shaken 25 times 1 foot in length within 7 seconds.
100 mL is filtered and rinsed with sterile distilled water 3 times.
The membrane filter is aseptically removed from filter holder.
A sterile padded petri dish is used and the membrane filter is rolled onto the pad making sure no air bubbles form.
The sterile labeled lid is placed on the petri dish.
2 blanks and a known is run with each series of samples.
The samples are placed in the $35^{o}C \pm .5^{o}C$ incubator stacked no higher than 3 for 22 – 24 hours (Humidity can be maintained by saturated paper towels placed under containers holding petri dishes.)
After 22- 24 hours view the petri dishes under a 10 –15 power magnification with cool white fluorescent light.
Count all colonies that appear pink to dark red with a metallic surface sheen – the sheen may vary in size from a pin head to complete coverage.
Report as Total Coliform per 100 mL.
If no colonies are present report as <1 coliform/100mL.

Anything greater than 1 is over the limit for drinking water for 2 samples taken 24 hours apart. Further investigation may be necessary – follow Standard Methods accordingly.

Photograph and Credits to Mary McPherson
Aran™ Aqua Analytical Laboratory Director.

Operators analyze sludge samples to improve wasting.



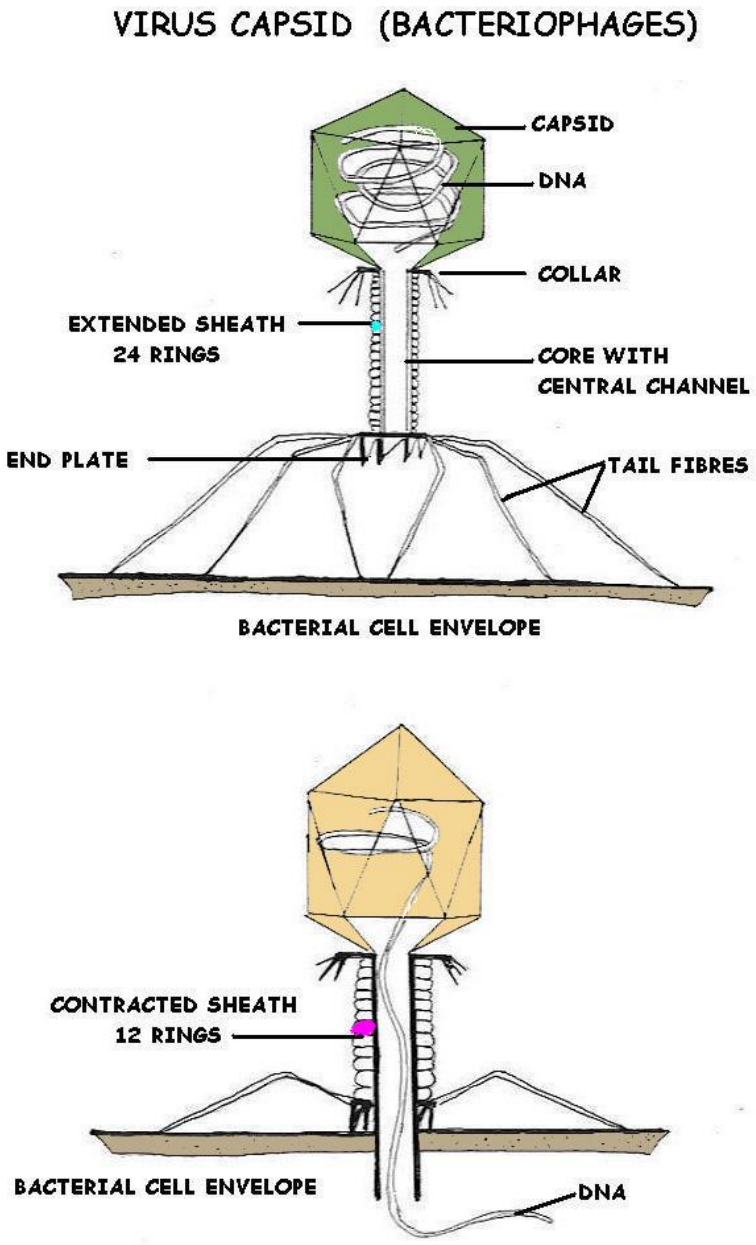
BACTERIOPHAGE

Bacteriophage

A bacteriophage (from 'bacteria' and Greek phagein, 'to eat') is any one of a number of viruses that infect bacteria. The term is commonly used in its shortened form, phage.

Typically, bacteriophages consist of an outer protein hull enclosing genetic material. The genetic material can be ssRNA (single stranded RNA), dsRNA, ssDNA, or dsDNA between 5 and 500 kilo base pairs long with either circular or linear arrangement. Bacteriophages are much smaller than the bacteria they destroy - usually between 20 and 200 nm in size.

Phages are estimated to be the most widely distributed and diverse entities in the biosphere. Phages are ubiquitous and can be found in all reservoirs populated by bacterial hosts, such as soil or the intestine of animals. One of the densest natural sources for phages and other viruses is sea water, where up to 9×108 virions per milliliter have been found in microbial mats at the surface, and up to 70% of marine bacteria may be infected by phages.

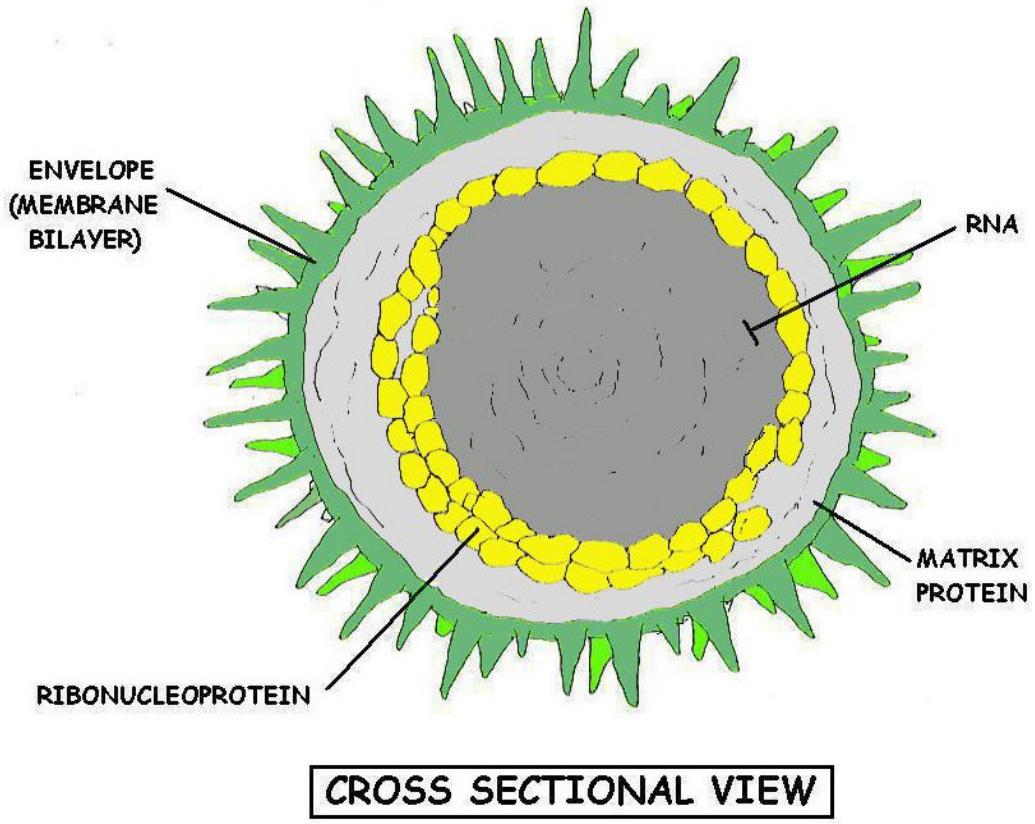


Release of Virions

Phages may be released via cell lysis or by host cell secretion. In the case of the T4 phage, in just over twenty minutes after injection upwards of three hundred phages will be released via lysis within a certain timescale. This is achieved by an enzyme called endolysin which attacks and breaks down the peptidoglycan. In contrast, "lysogenic" phages do not kill the host but rather become long-term parasites and make the host cell continually secrete more new virus particles. The new virions bud off the plasma membrane, taking a portion of it with them to become enveloped viruses possessing a viral envelope. All released virions are capable of infecting a new bacterium.

Viruses

Viruses are acellular microorganisms. They are made up of only genetic material and a protein coat. Viruses depend on the energy and metabolic machinery of the host cell to reproduce. A virus is an infectious agent found in virtually all life forms, including humans, animals, plants, fungi, and bacteria. Viruses consist of genetic material—either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)—surrounded by a protective coating of protein, called a capsid, with or without an outer lipid envelope. Viruses are between 20 and 100 times smaller than bacteria and hence are too small to be seen by light microscopy.



Viruses vary in size from the largest poxviruses of about 450 nanometers (about 0.000014 in) in length to the smallest polioviruses of about 30 nanometers (about 0.000001 in). Viruses are not considered free-living, since they cannot reproduce outside of a living cell; they have evolved to transmit their genetic information from one cell to another for the purpose of replication. Viruses often damage or kill the cells that they infect, causing disease in infected organisms. A few viruses stimulate cells to grow uncontrollably and produce cancers. Although many infectious diseases, such as the common cold, are caused by viruses, there are no cures for these illnesses. The difficulty in developing antiviral therapies stems from the large number of variant viruses that can cause the same disease, as well as the inability of drugs to disable a virus without disabling healthy cells. However, the development of antiviral agents is a major focus of current research, and the study of viruses has led to many discoveries important to human health.

Virions
Individual viruses, or virus particles, also called virions, contain genetic material, or genomes, in one of several forms. Unlike cellular organisms, in which the genes always are made up of DNA, viral genes may consist of either DNA or RNA. Like cell DNA, almost all viral DNA is double-stranded, and it can have either a circular or a linear arrangement. Almost all viral RNA is single-stranded; it is usually linear, and it may be either segmented (with different genes on different RNA molecules) or non-segmented (with all genes on a single piece of RNA).

Capsids
The viral protective shell, or capsid, can be either helical (spiral-shaped) or icosahedral (having 20 triangular sides). Capsids are composed of repeating units of one or a few different proteins. These units are called protomers or capsomers. The proteins that make up the virus particle are called structural proteins. Viruses also carry genes for making proteins that are never incorporated into the virus particle and are found only in infected cells. These viral proteins are called nonstructural proteins; they include factors required for the replication of the viral genome and the production of the virus particle.

Capsids and the genetic material (DNA or RNA) they contain are together referred to as nucleocapsids. Some virus particles consist only of nucleocapsids, while others contain additional structures.

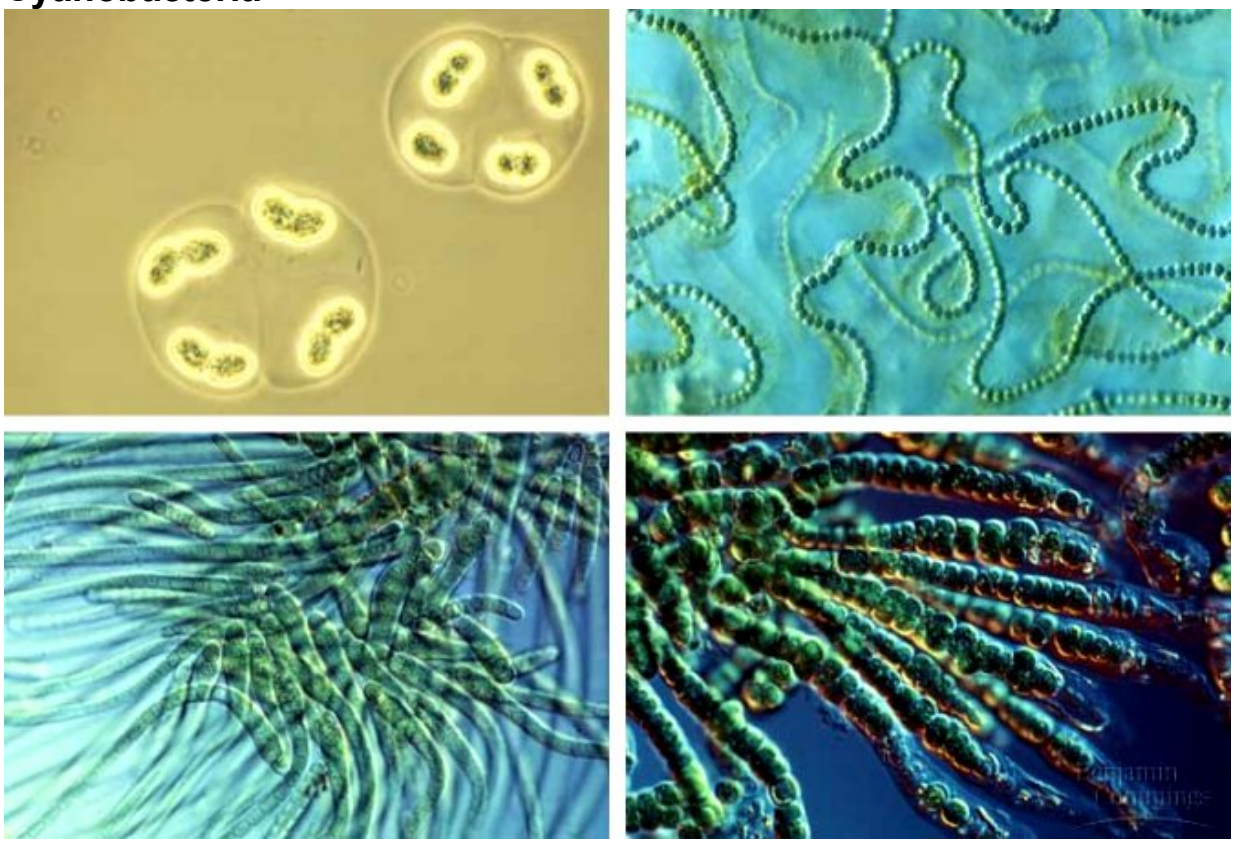
Some icosahedral and helical animal viruses are enclosed in a lipid envelope acquired when the virus buds through host-cell membranes. Inserted into this envelope are glycoproteins that the viral genome directs the cell to make; these molecules bind virus particles to susceptible host cells.

Bacteriophages
The most elaborate viruses are the bacteriophages, which use bacteria as their hosts. Some bacteriophages resemble an insect with an icosahedral head attached to a tubular sheath. From the base of the sheath extend several long tail fibers that help the virus attach to the bacterium and inject its DNA to be replicated, direct capsid production, and virus particle assembly inside the cell.

Viroids and Prions
Viroids and prions are smaller than viruses, but they are similarly associated with disease. Viroids are plant pathogens that consist only of a circular, independently replicating RNA molecule. The single-stranded RNA circle collapses on itself to form a rod-like structure. The only known mammalian pathogen that resembles plant viroids is the deltavirus (hepatitis D), which requires hepatitis B virus proteins to package its RNA into virus particles. Co-infection with hepatitis B and D can produce more severe disease than can infection with hepatitis B alone. Prions are mutated forms of a normal protein found on the surface of certain animal cells.

Virus Classification
Viruses are classified according to their type of genetic material, their strategy of replication, and their structure. The International Committee on Nomenclature of Viruses (ICNV), established in 1966, devised a scheme to group viruses into families, subfamilies, genera, and species. The ICNV report published in 1995 assigned more than 4000 viruses into 71 virus families. Hundreds of other viruses remain unclassified because of the lack of sufficient information.

Cyanobacteria



Cyanobacteria

Cyanobacteria, also known as blue-green algae, blue-green bacteria or Cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis. The name "cyanobacteria" comes from the color of the bacteria (Greek: kyanós = blue). They are a significant component of the marine nitrogen cycle and an important primary producer in many areas of the ocean, but are also found on land.

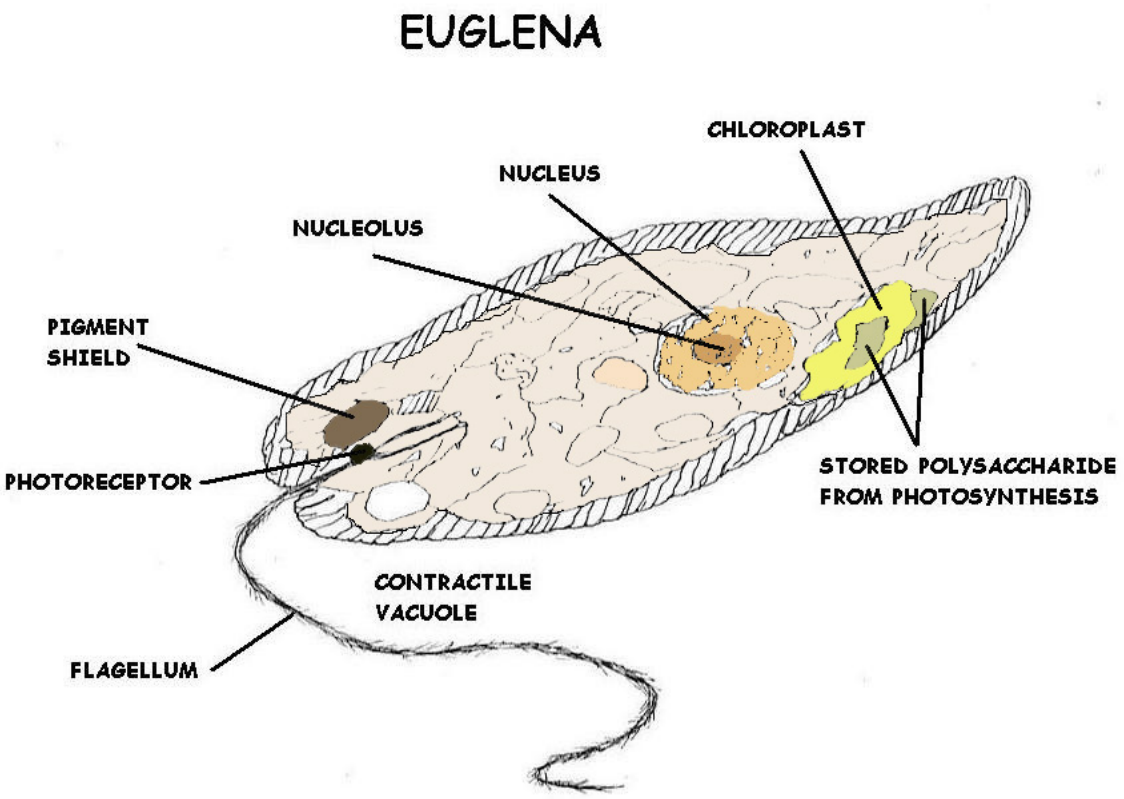
Cyanobacteria include unicellular and colonial species. Colonies may form filaments, sheets or even hollow balls. Some filamentous colonies show the ability to differentiate into several different cell types: vegetative cells, the normal, photosynthetic cells that are formed under favorable growing conditions; akinetes, the climate-resistant spores that may form when environmental conditions become harsh; and thick-walled heterocysts, which contain the enzyme nitrogenase, vital for nitrogen fixation. Heterocysts may also form under the appropriate environmental conditions (anoxic) wherever nitrogen is necessary. Heterocyst-forming species are specialized for nitrogen fixation and are able to fix nitrogen gas, which cannot be used by plants, into ammonia (NH_3), nitrites (NO_2) or nitrates (NO_3), which can be absorbed by plants and converted to protein and nucleic acids.

The rice paddies of Asia, which produce about 75% of the world's rice, could not do so were it not for healthy populations of nitrogen-fixing cyanobacteria in the rice paddy fertilizer too.

Many cyanobacteria also form motile filaments, called hormogonia, that travel away from the main biomass to bud and form new colonies elsewhere. The cells in a hormogonium are often thinner than in the vegetative state, and the cells on either end of the motile chain may be tapered. In order to break away from the parent colony, a hormogonium often must tear apart a weaker cell in a filament, called a necridium.

Each individual cell of a cyanobacterium typically has a thick, gelatinous cell wall. They differ from other gram-negative bacteria in that the quorum sensing molecules autoinducer-2[4] and acyl-homoserine lactones are absent. They lack flagella, but hormogonia and some unicellular species may move about by gliding along surfaces. In water columns some cyanobacteria float by forming gas vesicles, like in archaea.

TAKING A SAMPLE FROM A STREAM



Euglena

Euglenas are common protists, of the class Euglenoidea of the phylum Euglenophyta. Currently, over 1000 species of Euglena have been described. Marin et al. (2003) revised the genus so, and including several species without chloroplasts, formerly classified as Astasia and Khawkinea. Euglena sometimes can be considered to have both plant and animal features.

Euglena gracilis has a long hair-like thing that stretches from its body. You need a very powerful microscope to see it. This is called a flagellum, and the euglena uses it to swim. It also has a red eyespot. Euglena gracilis uses its eyespot to locate light. Without light, it cannot use its chloroplasts to make itself food. In order for Euglena gracilis to make more Euglena gracilis it will complete a process called mitosis. That means it can split itself in half and become two Euglena gracilis. It can only do this if it is well-fed and if the temperature is right. Euglena gracilis can reproduce better in warm temperatures.

Euglena gracilis, and other euglena, are harmless to people, but they are often signs that water is polluted, since they do well where there is a lot of green algae to eat. Green algae does well where there is a lot of nitrogen (comes from waste) in the water. If you don't clean your swimming pool, leaves and twigs get in the water and turn into waste. Then algae and euglena show up.

KINGDOM: Protist, PHYLUM: Euglenophyta, CLASS: Euglenophyceae, ORDER: Euglenales, FAMILY: Euglenidae, GENUS: Euglena, SPECIES: Euglena gracilis

Peptidoglycan

Peptidoglycan, also known as murein, is a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of eubacteria. The sugar component consists of alternating residues of β-(1,4) linked N-acetylglucosamine and N-acetylmuramic acid residues. Attached to the N-acetylmuramic acid is a peptide chain of three to five amino acids. The peptide chain can be cross-linked to the peptide chain of another strand forming the 3D mesh-like layer.

Hepatitis

There are five types of hepatitis -- A through E -- all of which cause inflammation of the liver. Type D affects only those who also have hepatitis B, and hepatitis E is extremely rare in the United States.

- Type A hepatitis is contracted through anal-oral contact, by coming in contact with the feces of someone with hepatitis A, or by eating or drinking hepatitis A contaminated food or water.
- Type B hepatitis can be contracted from infected blood, seminal fluid, vaginal secretions, or contaminated drug needles, including tattoo or body-piercing equipment. It can also be spread from a mother to her newborn.
- Type C hepatitis is not easily spread through sex. You're more likely to get it through contact with infected blood, contaminated razors, needles, tattoo and body-piercing equipment, or manicure or pedicure tools that haven't been properly sanitized, and a mother can pass it to her baby during delivery.
- Type D hepatitis can be passed through contact with infected blood, contaminated needles, or by sexual contact with an HIV-infected person.
- Type E hepatitis is most likely to be transmitted in feces, through oral contact, or in water that's been contaminated.

MATH CONVERSION FACTORS

1 PSI = 2.31 Feet of Water
1 Foot of Water = .433 PSI
1.13 Feet of Water = 1 Inch of Mercury
454 Grams = 1 Pound
1 Gallon of Water = 8.34 pounds/gal
1 mg/L = 1 PPM
17.1 mg/L = 1 Grain/Gallon
1% = 10,000 mg/L
694 Gallons per Minute = MGD
1.55 Cubic Feet per Second = 1 MGD
60 Seconds = 1 Minute
1440 Minutes = 1 Day
.746 kW = 1 Horsepower
1 + 1 = 2

LENGTH
12 Inches = 1 Foot
3 Feet = 1 Yard
5280 Feet = 1 Mile

AREA
144 Square Inches = 1 Square Foot
43,560 Square Feet = 1 Acre

VOLUME
1000 Milliliters = 1 Liter
3.785 Liters = 1Gallon
231 Cubic Inches = 1 Gallon
7.48 Gallons = 1 Cubic Foot
62.38 Pounds = 1 Cubic Foot

DIMENSIONS

SQUARE: Area (sq. ft.) = Length X Width

Volume (cu. ft.) = Length (ft) X Width (ft) X Height (ft)

CIRCLE: Area (sq. ft.) = 3.14 X Radius (ft) X Radius (ft)

CYLINDER: Volume (Cu. ft) = 3.14 X Radius (ft) X Radius (ft) X Depth (ft)

SPHERE: $\frac{(3.14)\ (\text{Diameter})^3}{(6)}$ Circumference = 3.14 X Diameter

GENERAL

POUNDS PER DAY AKA SOLIDS APPLIED =
Concentration (mg/L) X Flow (MG) X 8.34

PERCENT EFFICIENCY = $\frac{\text{In – Out}}{\text{In}}$ X 100

TEMPERATURE: $^0F = (^0C \times 9/5) + 32$
$^0C = (^0F - 32) \times 5/9$

CONCENTRATION: Conc. (A) X Volume (A) = Conc. (B) X Volume (B)

FLOW RATE (Q): Q = A X V (Quantity = Area X Velocity)

FLOW RATE (gpm): Flow Rate (gpm) = $\frac{2.83 \text{ (Diameter, in)}^2 \text{ (Distance, in)}}{\text{Height, in}}$

% SLOPE = $\frac{\text{Rise (feet)}}{\text{Run (feet)}}$ X 100

ACTUAL LEAKAGE = $\frac{\text{Leak Rate (GPD)}}{\text{Length (mi.) X Diameter (in)}}$

VELOCITY = $\frac{\text{Distance (ft)}}{\text{Time (Sec)}}$

N = Manning's Coefficient of Roughness
R = Hydraulic Radius (ft.)
S = Slope of Sewer (ft/ft.)

HYDRAULIC RADIUS (ft) = $\frac{\text{Cross Sectional Area of Flow (ft)}}{\text{Wetted pipe Perimeter (ft)}}$

WATER HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960}$

BRAKE HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960 \text{ X Pump Efficiency}}$

MOTOR HORSEPOWER = $\frac{\text{Flow (gpm) X Head (ft)}}{3960 \text{ X Pump Eff. X Motor Eff.}}$

MEAN OR AVERAGE = $\frac{\text{Sum of the Values}}{\text{Number of Values}}$

TOTAL HEAD (ft) = Suction Lift (ft) X Discharge Head (ft)

SURFACE LOADING RATE = $\frac{\text{Flow Rate (gpm)}}{\text{Surface Area (sq. ft)}}$
(gal/min/sq. ft.)

MIXTURE = $\frac{\text{(Volume 1, gal) (Strength 1, \%) + (Volume 2, gal) (Strength 2,\%)}}{\text{(Volume 1, gal) + (Volume 2, gal)}}$
STRENGTH (%)

INJURY FREQUENCY RATE = $\frac{\text{(Number of Injuries) 1,000,000}}{\text{Number of hours worked per year}}$

DETENTION TIME (hrs) = $\frac{\text{Volume of Basin (gals) X 24 hrs}}{\text{Flow (GPD)}}$

Slope = $\frac{\text{Rise (ft)}}{\text{Run (ft)}}$ Slope(%) = $\frac{\text{Rise (ft) X 100}}{\text{Run (ft)}}$

POPULATION EQUIVENT (PE):
1 PE = .17 Pounds of BOD per Day
1 PE = .20 Pounds of Solids per Day
1 PE = 100 Gallons per Day

LEAKAGE (GPD/inch) = $\frac{\text{Leakage of Water per Day (GPD)}}{\text{Sewer Diameter (inch)}}$

CHLORINE DEMAND (mg/L) = Chlorine Dose (mg/L) – Chlorine Residual (mg/L)

τQ = Allowable time for decrease in pressure from 3.5 PSU to 2.5 PSI
τq = As below

$\tau Q = (0.022)\ (d_1^2 L_1)/Q$ $\tau q = \frac{[\ 0.085]\ [(d_1^2 L1)/(d_1 L_1)]}{q}$

Q = 2.0 cfm air loss
θ = .0030 cfm air loss per square foot of internal pipe surface
δ = Pipe diameter (inches)
L = Pipe Length (feet)

$V = \frac{1.486}{\nu} R^{2/3} S^{1/2}$

V = Velocity (ft./sec.)
ν = Pipe Roughness
R = Hydraulic Radius (ft)
S= Slope (ft/ft)

HYDRAULIC RADIUS (ft) = $\frac{\text{Flow Area (ft. 2)}}{\text{Wetted Perimeter (ft.)}}$

WIDTH OF TRENCH (ft) = Base (ft) + (2 Sides) X $\frac{\text{Depth (ft 2)}}{\text{Slope}}$

AMPERAGE = $\frac{\text{Voltage}}{\text{Ohms}}$

VOLTAGE IMBALANCE = $\frac{\text{Maximum Voltage Deviation (Volts) X 100}}{\text{Average Voltage (Volts)}}$

LABORATORY

TSS (mg/L) = Paper Wt. And Dried Solids (g) – Paper Wt. (g) X 1,000,000
Milliliters of Sample

BOD (mg/L = (Initial DO – Final DO) X 300
(unseeded) Milliliters of Sample

LANGELIER INDEX = pH - pH_s

STABILIZATION PONDS

DETENTION TIME (Days) = Volume of Ponds (gals)
Flow Rate (gals/day)

ORGANIC LOADING (Lbs. Of BOD/Acre/Day) = Pounds of BOD Applied per Day
Surface Areas (Acres)

FIXED MEDIA

HYDRAULIC LOADING (gals/1000 cu. ft./day) = Flow Rate (gals./day)
1000's Cubic Feet of Media

ORGANIC LOADING (lbs BOD/day/1000 cu. ft.) = Pounds of BOD applied per Day
1000'S OF Cubic Feet of Media

ACTIVATED SLUDGE

DETENTION TIME (hrs.) = Volume of the tank (gals)
Flow Rate (gals/hour)

SVI (mg/L) = Settled Sludge Volume (mls) X 1000
MLSS (mg/L)

SDI (g/ml) = 1 X 100
SVI

F/M = BOD (applied to aerator) X Flow (MGD) X 8.34
Pounds of Solids under Aeration

MCRT (Days) = Pounds of Solids under Aeration B Pounds of Solids in Clarifier
Pounds of Solids Wasted B Pounds of Solids over the Weirs

DIGESTER AND SOLIDS HANDLING

OXYGEN UPTAKE RATE (OUR) = $\frac{\text{mg of } O_2 \text{ used}}{\text{Minute}}$

ORGANIC LOADING (lbs./day/cu. ft.) = $\frac{\text{Pounds of Volatile Solids applied per Day}}{\text{Volume of Digester (cu. ft.)}}$

VOLATILE SOLIDS REDUCTION = $\frac{(\text{In} - \text{Out})\ (100\%)}{\text{In} - (\text{In} - \text{Out})}$

DRY POLYMER (Lbs) = (Gal. Of solution) X (8.34 lbs./gal.) X (% polymer solution)

SLUDGE APPLICATION (lbs)=(Gal. Of Sludge) X (8.34 lbs./gal.) X (% Solids in sludge)

1 TON = 2,000 lbs I METER = 3.28 Feet

Volume in Cubic Feet

Cube Formula
V= (L) (W) (D)
Volume= Length X Width X Depth

Cylinder Formula
V= (.785) (D^2) (d)

Build it, Fill it and Dose it.

1. Convert 10 cubic feet to gallons of water.

There is 7.48 gallons in one cubic foot.

2. A tank weighs 800 pounds, how many gallons are in the tank?

3. Convert a flow rate of 953 gallons per minute to million gallons per day.
There is 1440 minutes in a day.

4. Convert a flow rate of 610 gallons per minute to million of gallons per day.

5. Convert a flow of 550 gallons per minute to gallons per second.

6. Now, convert this number to liters per second.

7. A tank is 6' X 15' x 7' and can hold a maximum of _____ gallons of water.
V= (L) (W) (D) X 7.48 =

8. A tank is 25' X 75' X 10' what is the volume of water in gallons?
V= (L) (W) (D) X 7.48 =

9. In Liters?
V= (L) (W) (D) X 7.48 =_____ X 3.785

10. A tank holds 67,320 gallons of water. The length is 60' and the width is 15'. How deep is the tank?

Gallons_____ ÷ 7.48 = _____ 60 X 15 =

11. The diameter of a tank is 60' and the depth is 25'. How many gallons does it hold?

Cylinder Formula
V= (.785) (D^2) (d)

.785 X 60' X 60' X 25' X 7.48 =

Cubic Feet Information
There is no universally agreed symbol but the following are used:
cubic feet, cubic foot, cubic ft
cu ft, cu feet, cu foot
ft^3, $feet^3$, $foot^3$
$feet^3$, $foot^3$, ft^3
feet/-3, foot/-3, ft/-3

Wastewater Treatment Production Math Numbering System
In water treatment, we express our production numbers in Million Gallon numbers. Example 2,000,000 or 2 million gallons would be expressed as 2 MG or 2 MGD. Hints. A million has six zeros, you can always divide your final number by 1,000,000 or move the decimal point to the left six places. Example 528,462 would be expressed .56 MGD.

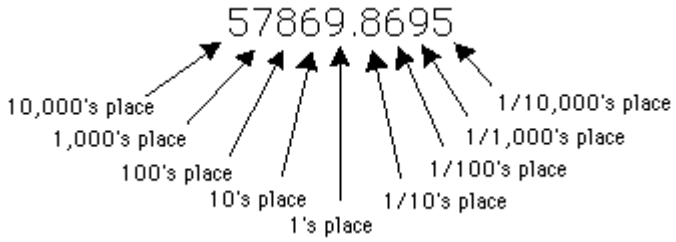
12. The diameter of a tank is 15 Centimeters or cm and the depth is 25 cm, what is the volume in liters?

2.54cm = 1 inch, 12 inches = 1 foot
15 cm ÷ 2.54 cm ÷ 12 inches = .492 feet

.785 X .492' X .492' X _____' =_____ X 7.48 = _____ X 3.785 L =

Percentage and Fractions
Let's look again at the sequence of numbers 1000, 100, 10, 1, and continue the pattern to get new terms by dividing previous terms by 10:

.1 = 1/10
.01 = 1/100
.001 = 1/1000



So just as the digits to the left of the decimal represent 1's, 10's, 100's, and so forth, digits to the right of the decimal point represent 1/10's, 1/100's, 1/1000's, and so forth.

Let's express 5% as a decimal. 5 ÷ 100 = 0.05 or you can move the decimal point to the left two places.

Changing a fraction to a decimal:

Divide the numerator by the denominator

A. 5/10 (five tenths) = five divided by ten:

```
   .5
  -----
10 ) 5.0
   5 0
  ----
```

So 5/10 (five tenths) = .5 (five tenths).

B. How about 1/2 (one half) or 1 divided by 2 ?

```
   .5
  -----
2 ) 1.0
   1 0
   ----
```

So 1/2 (one half) = .5 (five tenths)

Notice that equivalent fractions convert to the same decimal representation.

8/12 is a good example. 8 ÷ 12 =.66666666 or rounded off to .667

How about 6/12 or 6 inches? .5 or half a foot

Flow and Velocity

This depends on measuring the average velocity of flow and the cross-sectional area of the channel and calculating the flow from:

$Q(m^3/s) = A(m^2) \times V(m/s)$

Or

Q = A X V

Q CFM = Cubic Ft, Inches, Yards of time, Sec, Min, Hrs, Days
A = Area, squared Length X Width
V f/m = Inch, Ft, Yards, Per Time, Sec, Min, Ft or Speed

13. A channel is 3 feet wide and has water flowing to a depth of 2.5 feet. If the velocity through the channel is 2 fps or feet per second, what is the cfs flow rate through the channel?

Q = A X V

Q = 7.5 sq. ft. X 2 fps What is Q?
A= 3' X 2.5' = 7.5
V= 2 fps

14. A channel is 40 inches wide and has water flowing to a depth of 1.5 ft. If the velocity of the water is 2.3 fps, what is the cfs flow in the channel? **Q = A X V**
First we must convert 40 inches to feet.
40 ÷ 12" = 3.333 feet

A = 3.333' X 1.5' = 4.999 or round up to 5
V = 2.3 fps
We can round this answer up.

15. A channel is 3 feet wide and has a water flow at a velocity of 1.5 fps. If the flow through the channel is 8.1 cfs, what is the depth of the water?

Q = 8.1 cfs
V = 1.5 fps
A = ?

8.1 ÷1.5 = _____ Total Area

16. The flow through a 6 inch diameter pipe is moving at a velocity of 3 ft/sec. What is the cfs flow rate through the pipeline?

Q =
A = .785 X .5' X .5' =
V = 3 fps

17. An 8 inch diameter pipe has water flowing at a velocity of 3.4 fps. What is the gpm flow rate through the pipe?

Q = _____ cfs X 60 sec/min X 7.48 = _____ gpm
A = .785 X .667' X .667'
V = 3.4 fps

18. A 6 inch diameter pipe delivers 280 gpm. What is the velocity of flow in the pipe in ft/sec?

Take the water out of the pipe. 280 gpm ÷ 7.48 ÷ 60 sec/min = _____ cfs
Q =
A = .785 X .5' X .5' =
V =

19. A new section of 12 inch diameter pipe is to be disinfected before it is placed in service. If the length is 2000 feet, how many gallons of 5% NaOCl will be need for a dosage of 200 mg/L?

Cylinder Formula
$V = (.785) (D^2) (d)$

.785 X 1' X 1' X 2000' = _____ cu.ft. X 7.48 = _____ ÷ 1,000,000 = _____MG

Pounds per day formula = Flow (MGD) X Dose (mg/L) X 8.34 lbs/gal if 100% concentrate. If not, divide the lbs/day by the given %

0.0117436 MG X 200 mg/L X 8.34 =_____ lbs/day ÷ .05 =

20. A section of 6 inch diameter pipe is to be filled with water. The length of the pipe is 1320 feet long. How many kilograms of chlorine will be needed for a chlorine dose of 3 mg/L?

.785 X .5' X .5' X 1320' X 7.48 =_____ Make it MGD

Pounds per day formula = Flow X Dose X 8.34 X 45.4 Grams per pound

21. Determine the chlorinator setting in pounds per 24 hour period to treat a flow of 3.4 MGD with a chlorine dose of 3.35 mg/L?

Pounds per day formula = Flow (MGD) X Dose (mg/L) X 8.34 lbs/gal

22. To correct an odor problem, you use chlorine continuously at a dosage of 15 mg/L and a flow rate of 85 GPM. Approximately how much will odor control cost annually if chlorine is $0.17 per pound?

85 gpm X 1440 min/day = _____ gpd ÷ 1,000,000 = _____ MGD

_____ MGD X 15 mg/L X 8.34 lbs/gal X $0.17 per pound X 365 days/year =

23. A wet well measures 8 feet by 10 feet and 3 feet in depth between the high and low levels. A pump empties the wet well between the high and low levels 9 times per hour, 24 hours a day. Neglecting inflow during the pumping cycle, calculate the flow into the pump station in million of gallons per day (MGD).

Build it, fill it and do what it says, hint: X 9 X 24

24. A sewage treatment plant has a flow of 0.7 MGD and a BOD of 225 mg/L. On the basis of a national average of 0.2 lbs BOD per capita per day, what is the approximate population equivalent of the plant?

25. What is the detention time of a clarifier with a 250,000 gallon capacity if it receives a flow of 3.0 MGD?

DT= Volume in Gallons X 24 Divided by MGD

.25 MG X 24 hrs ÷ 3.0 MGD =_____ Hours of DT

Always convert gallons to MG

Crazy Math Section

The metric system is known for its simplicity. All units of measurement in the metric system are based on decimals—that is, units that increase or decrease by multiples of ten. A series of Greek decimal prefixes is used to express units of ten or greater; a similar series of Latin decimal prefixes is used to express fractions. For example, *deca* equals ten, *hecto* equals one hundred, *kilo* equals one thousand, *mega* equals one million, *giga* equals one billion, and *tera* equals one trillion. For units below one, *deci* equals one-tenth, *centi* equals one-hundredth, *milli* equals one-thousandth, *micro* equals one-millionth, *nano* equals one-billionth, and *pico* equals one-trillionth.

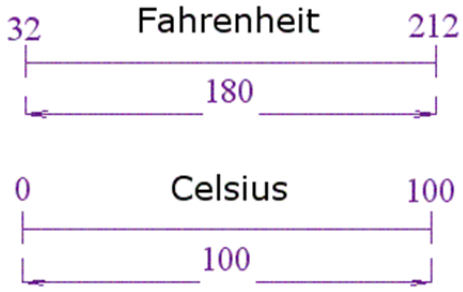
26. How many grams equal 4,500 mg?

Just simply divide by 1,000.

Temperature

There are two main temperature scales. The **Fahrenheit Scale** (used in the US), and the **Celsius Scale** (part of the Metric System, used in most other Countries)
They both measure the same thing (temperature!), just using different numbers.

- If you freeze water, it measures 0° in Celsius, but 32° in Fahrenheit
- If you boil water, it measures 100° in Celsius, but 212° in Fahrenheit
- The difference between freezing and boiling is 100° in Celsius, but 180° in Fahrenheit.



Conversion Method
Looking at the diagram, notice:

- The scales start at a different number (32 vs 0), so we will need to add or subtract 32
- The scales rise at a different rate (180 vs 100), so we will also need to multiply

And this is how it works out:

To convert from Celsius to Fahrenheit, first multiply by 180/100, then add 32

To convert from Fahrenheit to Celsius, first subtract 32, then multiply by 100/180

Note: 180/100 can be simplified to **9/5**, and likewise 100/180=**5/9**.
$^0F = (^0C \times 9/5) + 32$ 9/5 =1.8

$^0C = (^0F - 32) \times 5/9$ 5/9 = .555

27. Convert 20 degrees Celsius to degrees Fahrenheit.

$20^\circ \times 1.8 + 32 = F$

28. Convert 4 degrees Celsius to degrees Fahrenheit.

$4^\circ \times 1.8 + 32 = F$

Wastewater Treatment Filters

29. A 19 foot wide by 31 foot long rapid sand filter treats a flow of 2,050 gallons per minute. Calculate the filtration rate in gallons per minute per square foot of filter area.

GPM ÷ Square Feet

30. A 26 foot wide by 36 foot wide long rapid sand filter treats a flow of 2,500 gallons per minute. Calculate the filtration rate in gallons per minute per square foot of filter area.

Chemical Dose

31. A pond has a surface area of 51,500 square feet and the desired dose of a chemical is 6.5 lbs per acre. How many pounds of the chemical will be needed?

43,560 Square feet in an acre

51,500 ÷ 43,560 = _____ X 6.5 =

32. A pond having a volume of 6.85 acre feet equals how many millions of gallons?

33. Alum is added in a treatment plant process at a concentration of 10.5 mg/L. What should the setting on the feeder be in pounds per day if the plant is treating 3.5 MGD?

Pounds per day formula = Flow (MGD) X Dose (mg/L) X 8.34 lbs/gal

Q=AV Review

34. An 8 inch diameter pipe has water flowing at a velocity of 3.4 fps. What is the GPM flow rate through the pipe?
Q = 1.18 CFS x 60 Seconds x 7.48 GAL/CU.FT = 532 GPM
A = .785 X .667 X .667 X 1 = .349 Sq. Ft.
V= 3.4 Feet per second

35. A 6 inch diameter pipe delivers 280 GPM. What is the velocity of flow in the pipe in Ft/Sec?
280 GPM ÷ 60 seconds in a minute ÷ 7.48 gallons in a cu.ft. = .623 CFS

Q = .623
A = .785 X.5 X .5 =.196 Sq. Ft.
V = 3.17 Ft/Second

Collections

36. A 24-inch sewer carries an average daily flow of 5 MGD. If the average daily flow per person from the area served is 110 GPCD (gallons per capita per day), approximately how many people discharge into the wastewater collection system?

5,000,000 divided by 110 =

37. Using a dose rate of 5 mg/L, how many pounds of chlorine per day should be used if the flow rate is 1.2 MGD?

Pounds per day formula = Flow (MGD) X Dose (mg/L) X 8.34 lbs/gal

38. What capacity blower will be required to ventilate a manhole which is 3.5 feet in diameter and 17 feet deep? The air exchange rate is 16 air changes per hour.
.785 X 3.5' X 3.5' X 17' X 16 = _____ CFH

39. Approximately how many feet of drop are in 455 feet of 8-inch sewer with a 0.0475 ft/ft. slope?

SLOPE = $\frac{\text{Rise (ft)}}{\text{Run (ft)}}$ **SLOPE (%)** = $\frac{\text{Rise (ft) X 100}}{\text{Run (ft)}}$

455' X 0.0475 =

40. How much brake horsepower is required to meet the following conditions: 250 gpm, total head = 110 feet? The submersible pump that is being specified is a combined 64% efficient.

(250 X 110) ÷ (3960 X .64)

41. How wide is a trench at ground surface if a sewer trench is 2 feet wide at the bottom, 10 feet deep and the sides have been sloped at a 4/5 horizontal to 1 vertical (3/4:1) ratio?

(3/4:1) or 3 ÷ 4 = .75 X every foot of depth

42. A float arrives in a manhole 550 feet down stream three minutes and thirty seconds from its release point. What is the velocity in ft/sec.?
Velocity ft/sec = distance ÷ time

550' ÷ 3 **min stop convert min to sec. 3 X 60 = 180 + 30 = 210 sec**
550' ÷ 210 sec = _____ fps

43. A new sewer line plan calls out a 0.6% slope of the line. An elevation reading of 108.8 feet at the manhole discharge and an elevation of 106.2 feet at a distance of 200 feet from the manhole are recorded. What is the existing slope of the line that has been installed?

SLOPE = $\frac{\text{Rise (ft)}}{\text{Run (ft)}}$ **SLOPE (%)** = $\frac{\text{Rise (ft) X 100}}{\text{Run (ft)}}$

44. A triangular pile of spoil is 12 feet high and 14 feet wide at the base. The pile is 75' long. If the dump truck hauls 9 cubic yards of dirt, how many truck loads will it take to remove all of the spoil?

Given the base and the height of a triangle, we can find the area. Given the area and either the base or the height of a triangle, we can find the other dimension. The formula for area of a triangle is:

$A = \frac{1}{2} \cdot b \cdot h$ Or $A = \frac{b \cdot h}{2}$ where b is the base, h is the height.

14' X 12' ÷ 2 X 75' = _____ cu.ft. (27cuft/cuyrd)

45. A red dye is poured into an upstream manhole connected to a 12 inch sewer. The dye first appears in a manhole 400 feet downstream 3 minutes later. After 3 minutes and 40 seconds the dye disappears. Estimate the flow velocity in feet per second?
Velocity ft/sec = distance ÷ time
Make sure and convert time and average it.

46. Calculate the total dosage in pounds of a chemical. Assume the sewer is completely filled with the concentration. Pipe diameter: 18 inches, Pipe length: 420 feet, Dose: 120 mg/L.

Figure out the volume first.

.785 X 1.5' X 1.5' X 420' X 7.48 =_____ convert to MG

Pounds per day formula = Flow (MGD) X Dose (mg/L) X 8.34 lbs/gal

Answers

1. 7.48 X 10 = 74.8
2. 800 ÷ 8.34 = 95.92 gallons
3. 1372320 or 1.3 MGD
4. 610 X 1441 = 878400 or 0.87 MGD
5. 550 ÷ 60 = 9.167 gpm
6. 9.167 X 3.785 = 34.695 Liters
7. 630 Area 4712 gallons
8. 18,750 cu. ft. X 7.48 = 140250 gallons
9. 140250 X 3.785 = 530846 Liters
10. 10 feet deep
11. 528462 or .5 MG
12. 1.166 Gallons X 3.785 = 4.412 Liters
13. 15 cfs
14. 11.49 cfs
15. 1.8’
16. .58875 cfs
17. 533 gpm
18. 3.2 ft/sec
19. 46.9 gal
20. .002 kg
21. 94.9 lbs/day
22. $950.12
23. .387 MG
24. 6567.75
25. 2 hrs
26. 4.5 grams
27. 68° F
28. 39°F
29. 3.48 gpm/sq.ft.
30. 2.67 gpm/sq.ft.
31. 7.68 lbs
32. 2.231 MG
33. 306.495
34. 532 gpm
35. 3.2 fps
36. 45454.5 people
37. 50.04 lbs
38. 2615.6 cfh
39. 21.61 ft
40. 10.85 bhp
41. 17 ft
42. 2.62 fps
43. .013 or 1.3%
44. 26 trucks
45. 2 fps
46. 5.55 lbs

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