Intermediate Wastewater Laboratory

Course #2202

TENNESSEE DEPARTMENT OF ENVIRONMENT AND CONSERVATION

July 2012

Fleming Training Center

http://tn.gov/environment/fleming/

Fleming Training Center

Your Partner in Clean Water

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Intermediate Wastewater Lab

Course #2202

Instructor: Shannon Pratt

Week 1	Week 2
July 16-20, 2012	July 30-August 3, 2012

Monday, July 16:

8:30	Introductory Quiz
9:00	Introduction to Course (Lab Rules; Safety; Equipment; Sampling)
10:00	Dissolved Oxygen Analysis
11:00	Lunch
12:15	Biochemical Oxygen Demand (BOD)

Tuesday, July 17:

8:30	Approved Methods of Analysis; Review Sample NPDES Permit; Deficiencies at Wastewater Treatment Labs; Prep Filters for TSS;
11:00	Lunch
12:15	pH Analysis and Calibration

Wednesday, July 18:

8:30	Solids Analysis: Total Suspended Solids; Settleable Solids (Imhoff)
10:00	Whole Effluent Toxicity Testing
11:00	Lunch
12:15	Alkalinity
2:00	Solutions Chemistry

Thursday, July 19:

8:30	Read Results of TSS
9:30	Chemical Oxygen Demand (COD)
11:00	Lunch
12:15	Read COD's
12:45	Jar Testing
2:00	Turbidity

Friday, July 20:

8:30	Review
11:00	Lunch
12:15	Results of BOD Test and Lab Cleanup

Fleming Training Center

2022 Blanton Dr.
Murfreesboro, TN 37129

Phone: 615-898-6506
Fax: 615-898-8064
E-mail: Shannon.Pratt@tn.gov

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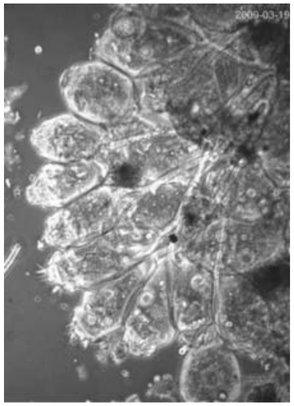
Week 2

July 30-August 3, 2012



Monday, July 30:

8:30	Chlorine Residual
11:00	Lunch
12:15	Testing for Fecal Coliforms and *E. coli*

Tuesday, July 31:

8:30	Nutrients; Ammonia Nitrogen (Sampling and Distillation)
11:00	Lunch
12:15	Ammonia Nitrogen Analyses
2:30	Results of Microbiological Testing

Wednesday, August 1:

8:30	Activated Sludge Process Control; Sensory Observation; Settleometer; Centrifuge Spin
11:00	Lunch
12:15	Oxygen Uptake Rate; Microscopic Examination of Activated Sludge

Thursday, August 2:

8:30	Nutrients: Nitrogen and Phosphorus
11:00	Lunch
12:15	QA/QC Program
1:00	Instrument and Probe Maintenance
2:00	Oil and Grease

Friday, August 3:

8:30	Activated Sludge Math
10:00	Course Review
11:00	Lunch
12:15	Exam and Course Evaluation

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E-mail: Shannon.Pratt@tn.gov

Intermediate Wastewater Laboratory Course

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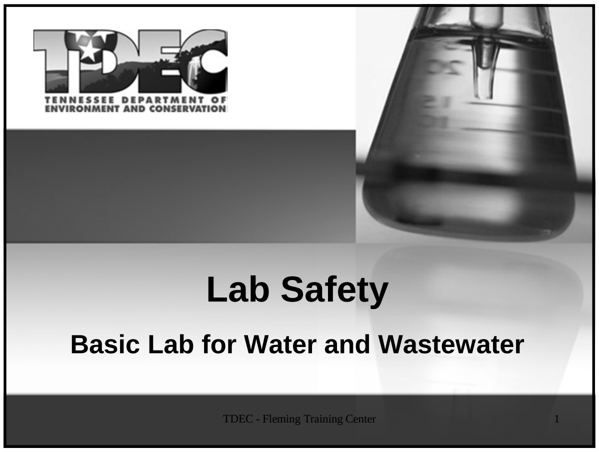
Section 1
Introduction

Lab Policies

1. No horse play.
2. No shorts or open-toed shoes.
3. No smoking, eating, dipping or drinking in the lab.
4. Put broken glass in broken glass container, NOT IN THE TRASH.
5. Do no pipet by mouth.
6. Each day after class:
    - All used glassware will be washed in hot soapy water, rinsed in tap water, then distilled water.
    - All counter top will be wiped clean with disinfectant.
    - Balance room must be clean.
7. Used pipets are placed in containers containing detergent immediately after use, tip up.
8. Acid spills must be cleaned up immediately.
9. Pipet bulbs must be cleaned immediately after overpipeting.
10. Wear safety glasses when performing any experiment.
11. Wear aprons in the lab at all times.
12. Wear gloves when performing any experiment or washing glassware.
13. Wash your hands before leaving the laboratory.
14. Know where the eye wash stations are located and how to use them.
15. Know where the emergency shower is and how to use it.
16. Know where each fire extinguisher is located and how to use them.
17. Read carefully the Material Safety Data Sheets for all chemicals used in the laboratory.



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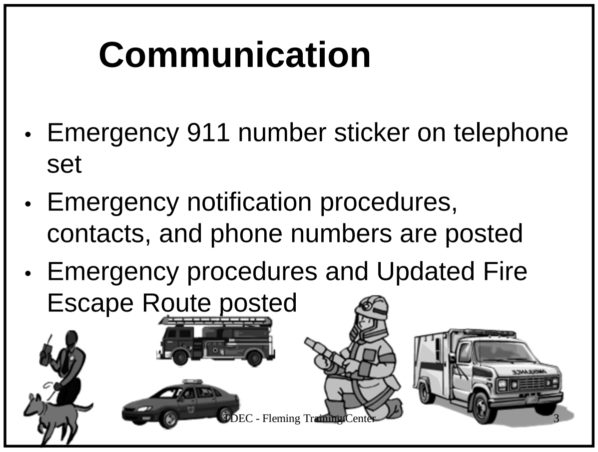
Lab Safety

Basic Lab for Water and Wastewater

Before Lab Work, Get to Know:

- Hazards of materials and their prescribed safety procedures
- Learn the lay-out of the building
  - location of emergency exits
  - emergency exit routes
  - emergency telephones
  - emergency ventilation system
  - fire-fighting equipment and how it works
  - emergency shower
  - first aid equipment

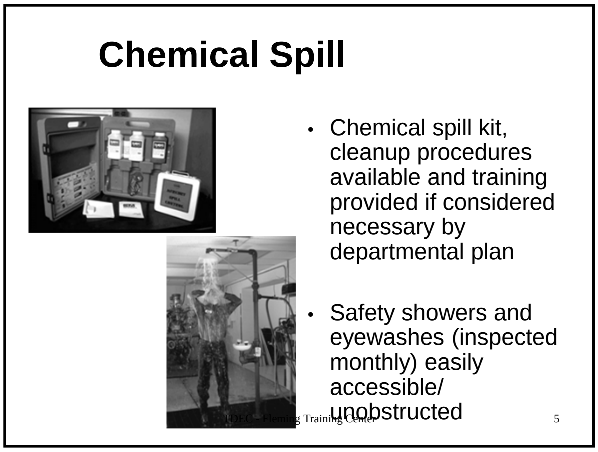


Communication

- Emergency 911 number sticker on telephone set
- Emergency notification procedures, contacts, and phone numbers are posted
- Emergency procedures and Updated Fire Escape Route posted

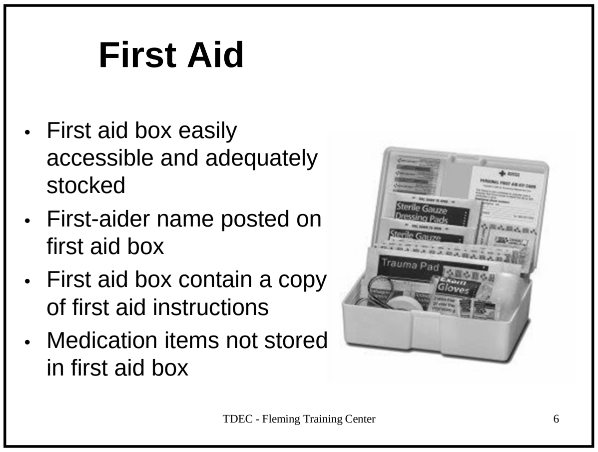
General Preparedness

- Emergency ventilation switch (in laboratory) unobstructed and function properly
- Exit signs readily visible/ illuminated
- Hazardous materials not stored along exit route and exit route free from obstruction
- Two well-separated exits, doors swing outward for chemistry laboratories or other high hazardous laboratories



Chemical Spill

- Chemical spill kit, cleanup procedures available and training provided if considered necessary by departmental plan
- Safety showers and eyewashes (inspected monthly) easily accessible/ unobstructed



First Aid

- First aid box easily accessible and adequately stocked
- First-aider name posted on first aid box
- First aid box contain a copy of first aid instructions
- Medication items not stored in first aid box

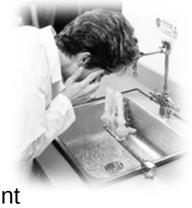
First Aid

- When a major injury occurs:
  - Call the emergency contact number, e.g. 911.
  - Keep the victim warm, lying down, and quiet until medical assistance arrives.
  - It is better NOT to move the injured person unless he or she is immediately threatened by further injury.

First Aid

- While waiting for the response personnel to arrive:
  - Treat acid and alkali burns with running water for 20-30 minutes
    - Use emergency eyewash/shower if necessary.
    - Do not attempt to neutralize.
    - Take care not to contaminate yourself.
  - Irrigate burned (heat or cryogenic) areas with cold water.

First Aid



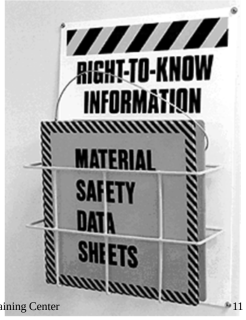
- While waiting for the response personnel to arrive:
  - Remove contact lenses, if present
    - Use eyewash for 15 minutes to cleanse eye after chemical splash.
  - Treat major bleeding with direct compression of the wound using a clean cloth.
  - Expose anyone who has inhaled toxic materials to fresh air.

Material Safety Data Sheets

- MSDS
- Keep on file for all chemicals purchased
  - According to the Americans with Disabilities Act of 1990, MSDS's should be kept for a minimum of 30 years
- Includes all information shown on a chemical label and more

Material Safety Data Sheets

- Also called MSDS
- Lists:
  - Common and chemical name
  - Manufacturer info
  - Hazardous ingredients
  - Health hazard data
  - Physical data
  - Fire and explosive data
  - Spill or leak procedures
  - PPE
  - Special precautions



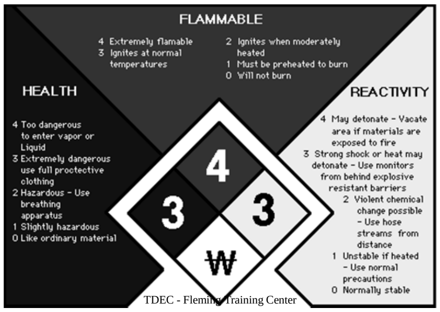
NFPA

- National Fire Protection Association
- Chemical hazard label
  - Color coded
  - Numerical system
    - Health
    - Flammability
    - Reactivity
  - Special precautions
- Labels are required on all chemicals in the lab

Degrees of Hazard

- Each of the colored areas has a number in it regarding the degree of hazard
  - 4 → extreme
  - 3 → serious
  - 2 → moderate
  - 1 → slight
  - 0 → minimal

Chemical Label



Health

- 4 (extreme) →highly toxic material
  - On very short exposure can cause death of major residual injury even with prompt medical treatment
  - A known/suspected human carcinogen, mutagen or teratogen
- 3 (serious) → toxic material
  - May cause serious temporary or residual injury on short term exposure even with prompt medical treatment
  - A known/suspected small animal carcinogen, mutagen or teratogen

Health

- 2 (moderate) → moderately toxic material
  - Intense or continued exposure could cause temporary incapacitation or possible residual injury even with prompt medical treatment
- 1 (slight) → slightly toxic material
  - May cause irritation but only minor residual injury even without treatment
  - Recognized innocuous material when used with responsible care
- 0 (minimal) → No chemical is without some degree of toxicity

Flammability

- 4 (extreme) → Extremely flammable
  - Flashpoint below 73°F
- 3 (serious) → Flammable
  - Vaporizes readily and can be ignited under almost all ambient conditions
  - May form explosive mixtures with or burn rapidly in air
  - May burn rapidly due to self-contained oxygen
  - May ignite spontaneously in air
  - Flash point at or above 73°F but less than 100°F

Flammability

- 2 (moderate) → Combustible
  - Must be moderately heated or exposed to relatively high temps for ignition to occur
  - Solids which readily give off flammable vapors
  - Flash point at or above 100 F but less than 200 F
- 1 (slight) → Slightly combustible
  - Must be preheated for ignition to occur
  - Will burn in air when exposed at 1500 F for 5 min
  - Flash point at or above 200 F
- 0 (minimal)
  - Will not burn
  - Will not exhibit a flash point
  - Will not burn in air when exposed at 1500 F for 5 min

Reactivity

- 4 (extreme)
  - Can explode or decompose violently at normal temperature and pressure
  - Can undergo a violent self-accelerating exothermic reaction with common materials or by itself
  - May be sensitive to mechanical or local thermal shock at normal temperature and pressure

Reactivity

- 3 (serious)
  - Can detonate or explode but requires a strong initiating force or confined heating before initiation
  - Readily promotes oxidation with combustible materials and may cause fires
  - sensitive to thermal or mechanical shock at elevated temp
  - May react explosively with water without requiring heat or confinement

Reactivity

- 2 (moderate)
  - Normally unstable and readily undergoes violent change but does not detonate
  - May undergo chemical change with rapid release of energy at normal temp and pressure
  - May react violently with water
  - Forms potentially explosive mixtures with water
- 1 (slight)
  - Normally stable material which can become unstable at high temperature and pressure
- 0 (minimal)
  - Normally stable material which is not reactive with water

Special

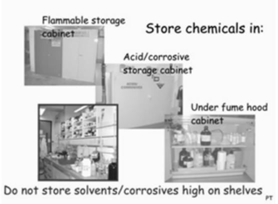
- W
  - Water reactive
- Ox
  - Oxidizing agent

Chemical Safety - Storage

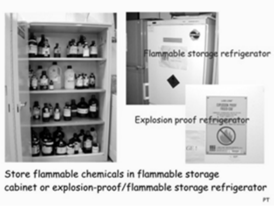
- Current chemical inventory available
- No expired chemicals.
  - Disposed of out-dated chemicals
- Chemical containers properly labeled, in good condition and closed properly
- Only compatible chemicals are stored together
  - Everything *not* stored in alphabetical order

Chemical Safety - Storage

- Secondary containment for stored chemicals as necessary.
  - Polyethylene trays for separate storage of acids and bases
- Chemicals stored at safe levels, in cabinets or on stable shelving (but not on high levels)



Chemical Safety - Flammables



- Stored in flammable cabinet and/or explosion-proof or flammable storage refrigerator
- Stored away from sources of heat and ignition
- Not stored along path of egress or in aisle space

Autoclave Safety

- To prevent bottles from shattering during pressurization, the caps of containers with liquids must be loosened before loading
- Before removing autoclaved items, wait 5 minutes for loads containing only dry glassware, and 10 minutes for autoclaved liquid loads
- Remember: these are not used to steam your lunch!!

Autoclave Safety

Lab Hygiene

- Food and drink are not to be stored or prepared in laboratories or chemical storerooms
- Use appropriate personal protective equipment and wash your hands regularly when working with chemical reagents, especially before meals or snacks.
- Smoking in laboratories is prohibited.

Lab Hygiene

- Loose sleeves are a hazard and should not be worn in the lab.
- If you have long hair, ensure that it is properly tied back.
- Wearing of contact lenses in the lab is strongly discouraged.
  - If it is unavoidable, advise your supervisor and co-workers so that this information is known in the event of a chemical splash in the eyes.

Personal Practices

- No inappropriate clothing and shoes (shorts, sandals, slippers, etc.)
- Gloves removed before handling telephone, door handle or leaving laboratory
- No pipeting by mouth

Personal Practices

- Lab coats and safety glasses/ goggles worn by all where necessary
- Proper gloves are used as needed
- Other personal protective equipment used properly as needed

Safety Glasses

- Unbreakable lenses of plastic or tempered glass
- For light-to-moderate work
- Can be prescription glasses
- Do not interfere with contact lenses

Goggles

- Work with significant risk of splash of chemicals or projectiles
- Can be worn over prescription glasses

Face Shield

- Work with significant risk of splash on face or possible explosion
- Face shield protects face adequately but not eyes
  - Should be worn with safety glasses and/or goggles to protect eyes

Pregnancy

- Women who are pregnant should discuss their work assignments with their supervisors to seek alternate work assignments if the potential for exposure to teratogens exist
  - Teratogens are reproductive toxins that may cause damage to the fetus
  - THM Plus method by Hach for determining Trihalomethanes deals with chloroform, a teratogen

Fume Hood

- All work generating toxic/hazardous vapor, fume, or aerosol performed in hood
- Front sash at appropriate level when hood is in use/ not in use
- Storage within the hood minimized and containers kept sealed

Fume Hood

- Verify that the fume cupboard is working properly.
- Locate work at least 6 inches inside the hood.
- Do not block the face of the hood, e.g. with shielding or large equipment.
- Do not block the space between tapered metal front lip and the work surface.
- Do not block rear exhaust slot. Place bulky items to rear and sides.
- Secure papers and other light weight materials to prevent their entrainment in the exhaust.

Lab Work Area

- The work bench is to be kept clean at all times, and free from chemicals and apparatus which are not required.
- Before starting an experiment, make sure you are familiar with all the procedures and the potential hazards of the starting materials and products.
  - Determine the appropriate safeguards and remedies.
  - Know the procedures for emergency shut off as well as the person and phone numbers to contact in case of emergency.
  - If anything unexpected occurs during your experiment, or if you are in any doubt, consult your supervisor immediately.

Handling Glassware

- Examine all glassware before use.
  - Discard any broken glass apparatus in the appropriate sharps container.
- Never store damaged glassware in cupboards.
  - Damaged glassware should either be sent for repair properly or disposed in a separate labeled container for sharps disposal.
- Use gloves when sweeping up broken glass, do not use bare hands.
  - Pick up fine glass particles with wet paper toweling.

Handling Glassware

- Cut ends of glass rods and tubing should always be fire-polished before use.
- Use a cloth for protection when inserting glass tubing, rods or thermometers into bungs or tubing
  - Use a lubricant or water where necessary.

Chemical Spills

- Small spills (generally less than 100 mL) can usually be cleaned up safely by the employees involved.
- The hazardous properties of the material must be considered when deciding whether it is a “small” spill or not, and therefore whether unassisted clean-up should be attempted.
- Employees must be trained in advance to handle cleanup of even small spills

American Development Corporation
821 William D. Jones Blvd, Fayetteville TN 37334
888-542-8561 Fax - 931-438-2673
www.adc-chem.com

Material Safety Data Sheet

This Material Safety Data Sheet conforms to the requirements of ANSI z400.1.
This MSDS complies with 29 CFR 1910.1200 (Hazard Communication Standard)
Read this MSDS before handling & disposing of this product.

Section 1: Material Identification and Company Identification

Product Identity:	Chlorine
Distributed By:	American Development Corporation
Address:	821 William D. Jones Blvd.
City/State:	Fayetteville TN 37334
Phone:	888-542-8561
Chem-Tel	800-255-3924

Section 2: Hazardous Ingredients

Component	CAS NO.	Concentration	Hazard
Chlorine	7782-50-5	99%	Corrosive

Section 3: Hazards Identification

Primary routes of exposure: Inhalation, skin or eye contact,

Potential Health Effects:

Eyes: Can cause severe burns and permanent damage to eyes.

Skin: Corrosive to the skin and all living tissue.

Ingestion: Unlikely. Gas at room temperature

Inhalation: Corrosive and irritating to the upper and lower respiratory tracts

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Section 4: First Aid Measures

Eye Contact:	Flush with copious quantities of water. Part eyelids to assure complete flushing. Continue for 15 minutes. Seek immediate medical attention.
Skin Contact:	Remove contaminated clothing. Flush affected area with copious quantities of water. Seek medical attention.
Ingestion:	Not likely
Inhalation:	Seek immediate medical attention. Rescue personal should wear SCBA. Medical treatment should be symptomatic and supportive.

Section 5: Fire Fighting Measures:

Extinguishing Media: Non-flammable. Use media for surrounding materials.

Hazardous combustion products:		None
Sensitivity to mechanical shock:		None
Sensitivity to static discharge:		None
NFPA Rating:	Health	3
	Fire	0
	Reactivity	0
	Hazard	Corrosive

Section 6: Accidental Release Measures:

Evacuate all personal from affected area.
Only properly trained personnel equipped with protective clothing
and respiratory protection should locate and stop release.

Section 7: Handling and Storage:

Secure cylinder to protect from falling.
Store in well ventilated areas.
Keep protective cap on cylinder when not in use.

Section 8: Exposure Controls/Personal Protection

Control Measures: Provide adequate general and local exhaust ventilation to maintain concentration well below exposure limits.

Recommended Personal Protective Equipment:

Respirator: In case of leakage, use an approved self-contained breathing apparatus.

Eyes: Safety glasses or goggles.

Gloves: Impervious, resistant gloves

Clothing: Impervious coveralls, boots and/or other resistant clothing.

Other protective measures: Emergency eye wash and safety showers for first aid should be available near work area.

Section 9: Physical Properties

Appearance:	Yellow or green	**Specific Gravity**	Gas
Physical State:	Gas	**Boiling Point:**	-31º F
Odor:	Suffocating pungent odor	**Freezing Point:**	- 150º F
Odor Threshold:	0.08 - 0.4 ppm	**Flash Point**	n/a

Section 10: Stability and Reactivity

Stability:	Stable under normal conditions
Polymerization:	Will not occur
Conditions to avoid:	Storage in poorly ventilated areas. Storage near a heat source.
Incompatible materials:	Chlorine reacts with reducing agents and combustibles. Materials such as acetylene, turpentine, hydrocarbons, ammonia, ether, must be kept away.
Hazardous decomposition products:	Chlorine will react with steam and water to give HCI fumes.

Section 11: Toxicological Information

Medical conditions aggravated by exposure:

Heart conditions
Asthma

Section 12: Ecological Information

Chlorine is harmful at uncontrolled quantities to aquatic life. Keep out of water. Releases as a gas may damage green vegatation and may be hazardous to birds and mammals.

Section 13: Disposal Considerations

Subject to disposal regulations: U.S. EPA 40 CFR 262.
Hazardous Waste number D001.
Dispose in accordance with all applicable regulations.

Section 14: Transportation Information

D.O.T. Proper Shipping Name: Chlorine

D.O.T. Hazard Class: 2.3 (poison)
Hazard zone B
UN number: 1017
D.O.T. Labels: **Primary:** Poison Gas **Subsidiary:** Corrosive
D.O.T. Placards: Poison Gas

2004 Emergency Response Guidebook Number 124

Section 15: Regulatory Information

TSCA Inventory Status: Yes
SARA - 313 Listed Chemicals: Yes

CERCLA: Yes
Reportable Quantity: 10 lbs

Section 16: Other Information

CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
IARC	International Agency for Research on Cancer
MSHA	Mine Safety and Health Administration
NIOSH	National Institute for Occupational Safety and Health
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL-C	OSHA Permissible Exposure Limit-OSHA Ceiling Exposure Limit
RCRA	Resource Conservation and Recovery Act
SARA	Superfund Amendments and Reauthorization Act of 1986
TLV-TWA	Threshold Limit Value - Time Weighted Average
TSCA	Toxic Substances Control Act

The information appearing in this document is based upon data obtained from the product manufacturer and/or supplier. While the information is believed to be pertinent and accurate, no warranty expressed or implied is given to it's accuracy. This MSDS is to be used as a guideline for safe work practices and emergency response.

ChemSafe 2000: Understanding MSDS (11 min video)

1. What does MSDS stand for?
2. List the sections of MSDS:
   - Chemical or __________ Name
   - Physical Data
     - __________ Point
     - __________ Pressure
     - __________
     - Solubility
     - __________
     - Odor
   - __________ Ingredients - any substance the chemical contains that may be hazardous, since some chemicals can be a mixture of more than one ingredient, the names of all the __________ __________ will be listed.
   - Fire & __________ Hazards – lists the circumstances under which the chemical will explode or catch fire, also lists appropriate ____________
   - __________ Data – lists what conditions to avoid to keep dangerous chemical reactions from happening
   - __________ or Leak Procedures – how to clean up or contain a __________ and how to __________ of chemical after spill
   - __________ Hazard Data – lists what exposures can pose a threat to your health and how it may effect or get into your __________
   - Special __________ - lists what gear (or PPE) you should wear
   - Special __________ - extra lists warning of handling, use in storage and recommended labeling
3. The name on the __________ will be the name under which you will find the MSDS.

ChemSafe 2000: Understanding MSDS (11 min video)

1. What does MSDS stand for? ***Material Safety Data Sheet***

2. List the sections of MSDS:

   - Chemical or ***Trade*** Name
   - Physical Data
     - ***Boiling*** Point
     - ***Vapor*** Pressure
     - ***Density***
     - Solubility
     - ***Appearance***
     - Odor
   - ***Hazardous*** Ingredients - any substance the chemical contains that may be hazardous, since some chemicals can be a mixture of more than one ingredient, the names of all the ***hazardous ingredients*** will be listed.
   - Fire & ***Explosion*** Hazards – lists the circumstances under which the chemical will explode or catch fire, also lists appropriate ***extinguishers***
   - ***Reactivity*** Data – lists what conditions to avoid to keep dangerous chemical reactions from happening
   - ***Spill*** or Leak Procedures – how to clean up or contain a ***spill*** and how to ***dispose*** of chemical after spill
   - ***Health*** Hazard Data – lists what exposures can pose a threat to your health and how it may effect or get into your ***body***
   - Special ***Protection*** - lists what gear (or PPE) you should wear
   - Special ***Precautions*** - extra lists warning of handling, use in storage and recommended labeling

3. The name on the ***label*** will be the name under which you will find the MSDS.

Laboratory Equipment

Identification, Handling and Cleaning

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Objectives

- Identify equipment commonly used in water treatment and wastewater laboratory
- Discuss accuracy and use of glassware
- Discuss how to maintain analytical equipment

Beakers

- Used for:
  - Mixing
  - Measuring approximate volumes
  - ~10% accuracy

Graduated Cylinders

- Accurate to ~1%
- Measures liquid volumes more accurately than beakers, but still not the most accurate
- Measure quicker

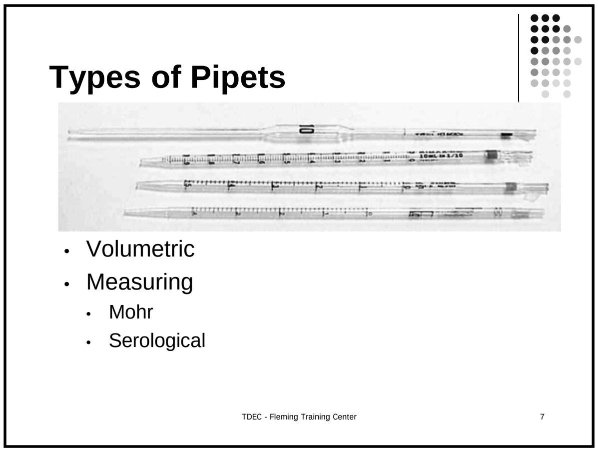
Volumetric Flasks

- Most accurate way to measure volume
- Disadvantage:
  - Only can measure one volume
  - Not used for storing or heating solutions

What Are Pipets?

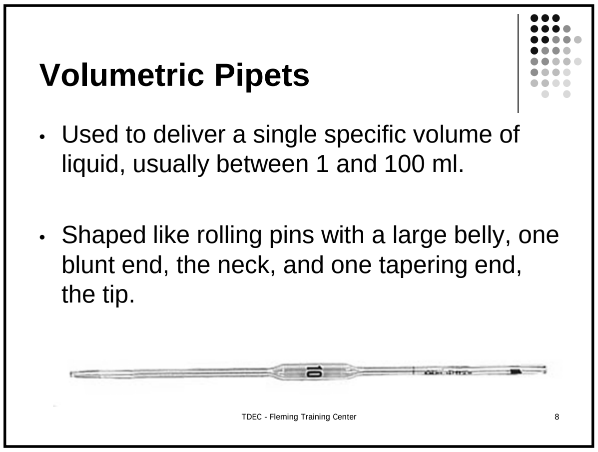
- Pipets are glass or plastic tubes, usually open at both ends,which are used to transfer specific amounts of liquid from one container to another.

- They are usually used for volumes between 1 and 100 milliliters.



Types of Pipets

- Volumetric
- Measuring
  - Mohr
  - Serological



Volumetric Pipets

- Used to deliver a single specific volume of liquid, usually between 1 and 100 ml.
- Shaped like rolling pins with a large belly, one blunt end, the neck, and one tapering end, the tip.

Volumetric Pipets

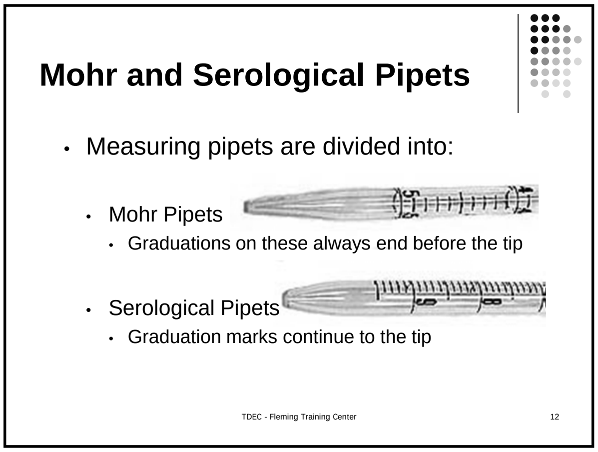
- Used for accurate measurements, since it is designed to deliver only one volume and is calibrated at that volume.
- Should be used when accuracy and reproducibility are crucial, because these can achieve accuracy to four significant figures.

Specifications on a Volumetric Pipet

- When emptying a volumetric pipet, the liquid is allowed to drain out
  - It is NOT forced out.
- After it is emptied, the small amount of liquid which remains in the tip should not be blown out.
- Volumetric pipets are NOT blow-out pipets

Measuring Pipets

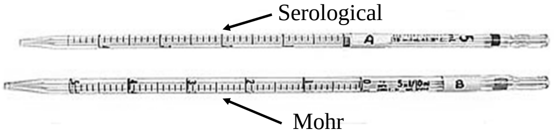
- They are straight glass or plastic tubes with one tapering end.
- Calibrated into small divisions so that various amounts of liquid can be measured with the same pipet.
- Usually used to measure any amount between 0.1ml and 25.0ml.
- They are not as accurate due to the fact that any imperfection in their internal diameter will have a greater effect on the volume delivered.



Mohr and Serological Pipets

- Measuring pipets are divided into:
  - Mohr Pipets
    - Graduations on these always end before the tip
  - Serological Pipets
    - Graduation marks continue to the tip

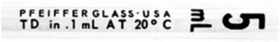
Examine pipets A and B.
Which is the serological and which is the Mohr?



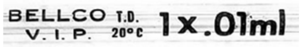
Specifications on a Measuring Pipet

- Maximum volume of liquid that can be transferred
- Size of the divisions on the pipet
- Temperature at which calibrations were made
- If the pipet is a “to deliver”(TD) or “to contain”(TC) pipet.

5 in 1/10 ml
TD 20oC



- Specifications on a pipet as shown above indicate that the pipet is calibrated in 1/10ml divisions and will deliver up to 5.0 ml within published tolerance levels at 20 C.

1 ml in1/100
TD 20oC

- These specifications indicate that the pipet is calibrated in 1/100 ml divisions and it will deliver up to 1.00 ml within published tolerance levels at 20 C.

Handling and Disposing of Pipets

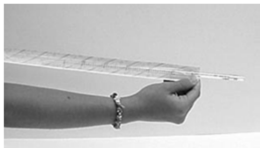
- Chipped and cracked pipets should be replaced as they are unsafe and may affect the accuracy of measurements.
- NEVER mouth pipet.
- Hold the pipet by the upper third of the tube and keep the tip from touching anything.

Handling and Disposing of Pipets

- Dispose dirty pipets by placing in soapy water solution in a tray or pipet washer.
- Place disposable pipets in a cardboard holder.
- Do not leave pipets on counters or sinks.

Handling Sterile Pipets

- When using sterile pipets, be sure to use proper sanitary techniques.
- If you have a sterile package of disposable pipets, tear only a small corner of the package open and push one pipet out of this opening, then immediately close the package to prevent contamination.



Handling Sterile Pipets

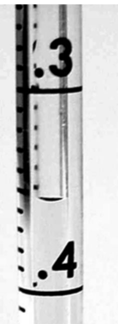
- If you are using sterile pipets in a pipet canister, place the canister on its side, slide off the cover, pull out one pipet and replace the cover immediately.

Transferring a Precise Volume of Liquid

- A pipet bulb is used to draw liquid up into the pipet.
- There are many types of pipet bulbs.

Transferring a Precise Volume of Liquid

- You should observe the meniscus at eye-level
- Touch the tip of the pipet to the inside of the container when the meniscus is at the desired level



Transferring a Precise Volume of Liquid

- Squeeze bulb and touch it to the mouth of the pipet.
- Place other end of the pipet in liquid to be transferred and slowly release pressure on bulb.
- Draw liquid up past desired level, quickly replacing bulb with index finger.

Transferring a Precise Volume of Liquid

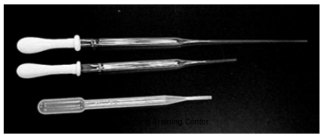
- Let liquid drain until bottom of meniscus lines up with desired level on pipet.
- Touch tip of pipet to inside of beaker to remove any adhering drops.
- Transfer liquid to second beaker and touch tip to inside of beaker and let liquid drain out of pipet.

Other Pipet Bulbs

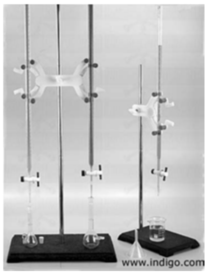
- Other pipet bulbs that are often used include the Vadosa pipet filler, seen on the left, and the pipet Pumper, on the right.

Other Pipet Types

- Transfer of uncalibrated volumes up to 2.5 ml can be accomplished using glass “transfer” or “Pasteur” pipets shown below. These may be sterilized before use.
- Roughly calibrated volumes of 1 and 2 ml can be transferred with the one piece plastic transfer pipets which may be purchased as sterile or non-sterile units.



Burettes and Titrations



- Burettes
  - Used for titrations
  - Treat like a Mohr pipet, do not let liquid completely drain out
  - Also, make sure to remove air bubble in tip before titrating

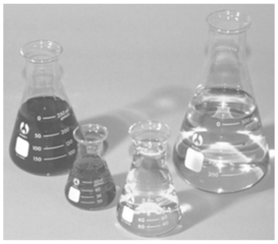
Flasks

- Distilling Flask

- Florence (Flat Bottom) Flask

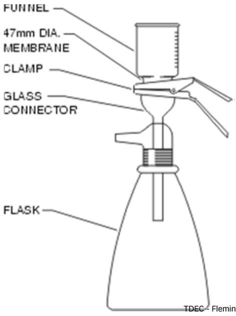
Flasks

- Erlenmeyer Flasks



- Filter Flask

Filter Apparatus



- Vacuum Pump

Bottles

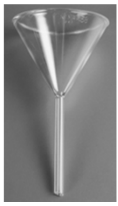
- Dilution Bottle
- Sample Bottles

Bottles

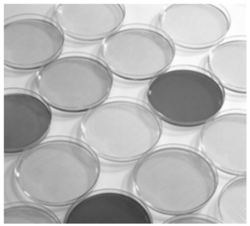
- Reagent Bottle
- Weighing Bottles

Funnels

- Separatory
- Buchner
- General



- Petri Dish
  - Culturing container
- Desiccators
  - Dust and moisture free



- Evaporating Dish
- Crucible

Centrifuge

- Used to separate materials of different density

Autoclave

- Pressure cooker used to sterilize glassware, bottles, membrane filter equip, culture media and contaminated material to be discarded.
- Standard temperature is set at121°C and 15 PSI

Refrigerators

Explosion proof

Walk-in cooler

- Sample storage should maintain between 1-5 °C
- Never store samples and chemicals together

Incubators

- Artificially heated container used for growing bacteria cultures
- Dry-Heat types hold temperatures to ± 0.5°C
- For E. coli and Total coliform = 35 ± 0.5°C
- Water Bath for fecals = 44.5 ± 0.2°C

Incubators

- For BOD incubation at 20 ± 1 °C
- Do not store chemical solutions and samples in same refrigerator

UV Sterilizer

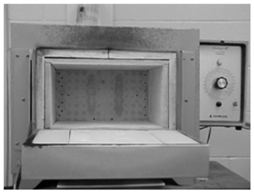
- Use in Bac'T Lab to sterilize test equipment

Drying Oven

Used more often in wastewater labs

For solids testing set oven at 103-105°C

Muffle Furnace



- High temp oven used to ignite or burn solids.
- Usually operate at temps of 550°C.
- More often used in Wastewater lab work.

Fume Hood

- Can prevent serious accidents
- Use whenever heat is used in a test procedure
- Fumes vented out of lab
- Use when harmful smoke, gas, vapors, splashes or fumes are possible

Water Still

- Produces distilled water for lab tests and rinsing washed glassware
- Removes dissolved minerals, organic and inorganic nonvolatile compounds
- Does not sterilize

Heating and Stirring Samples

- Combo Heat/Stir Plate
  - Can be used to stir or heat and stir samples
  - Safer than heating with an open flame
- Gas Burner
  - Uses natural gas

Balances

- Top Loading
  - Weighs to the nearest 0.01 g
- Analytical
  - Precise to 0.0001 g

pH Meter

- Use buffer solutions to calibrate
- Store electrodes properly
- Calibrate daily
- Maintain records on daily calibrations

Spectrophotometer

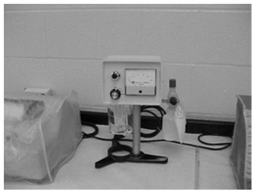
- HACH DR 4000
  - Factory pre-set programs for lab chemical analysis
- Very versatile

Colorimeters

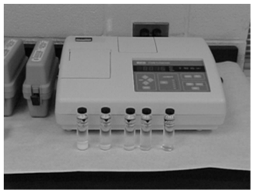
- Determine the concentration of many chemicals
- Most commonly used is chlorine type colorimeter
- Portable and battery powered

Amperometric Titrator

- Chlorine analysis
- Accurate and unaffected by sample color or turbidity
- Takes greater skill to use than DPD method with colorimetric devices



Turbidimeter



- Desk top and continuous on-line monitoring
- Position away from direct sunlight and have extra light bulb on hand
- Ensure sample bottles maintained; no scratches; acid clean if necessary

Chemical Storage

- Do not store volatile chemicals together
- Have separate storage cabinets for acids and bases/caustics

Flammable Cabinet

- Flammable chemicals should be kept in a flammable cabinet.

Safety Equipment

<u>PPE (Personal Protective Equipment):</u>

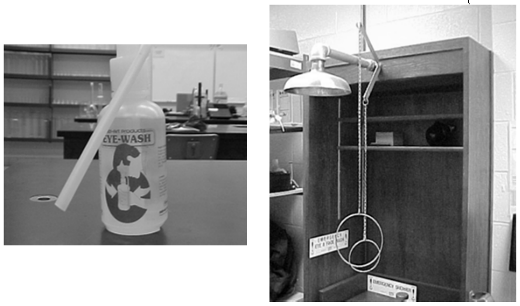
Goggles

Gloves

Aprons

Wear safety clothing.

Eye Wash and Shower



Cleaning Glassware

- Just because it looks clean does not mean residues are not left behind
- Results need to be accurate to use data for process control and/or reporting to the State
- Detergents, such as Alconox, may be sufficient
  - Should be phosphate-free

- Residues of minerals and other substances can build up on glassware, causing erroneous test results

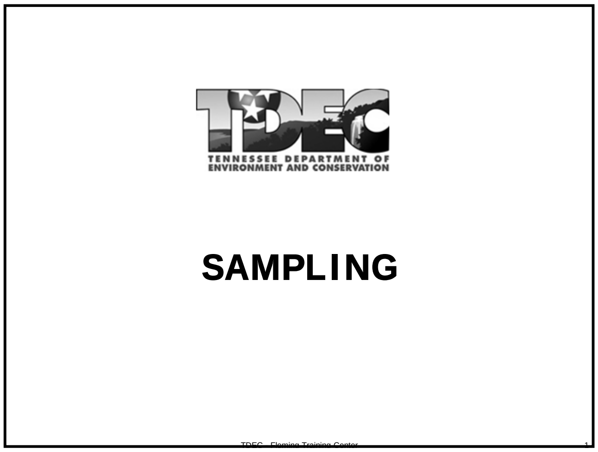
Steps for Washing

- Clean glassware using laboratory detergent (phosphate-free)
- Rinse with tap water
- Rinse at least three times with distilled water
- Let air dry

Steps for Acid-Washing

- Clean glassware using laboratory detergent (phosphate-free)
- Rinse with tap water
- Rinse with 1:1 hydrochloric acid or nitric acid
  - 1:1 means equal parts distilled water and acid
- Rinse well with distilled water
- Let air dry

Note: always use gloves and goggles when handling acids



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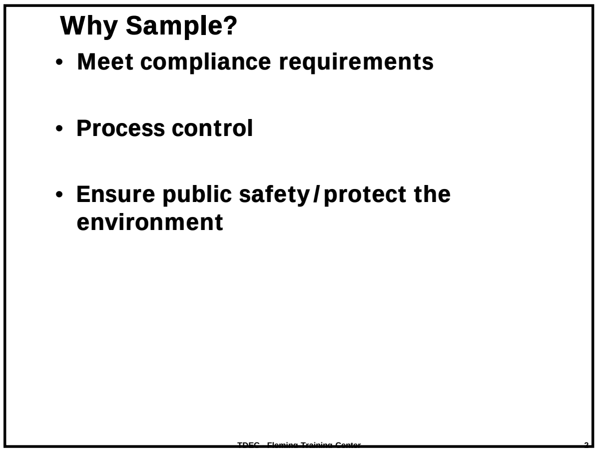
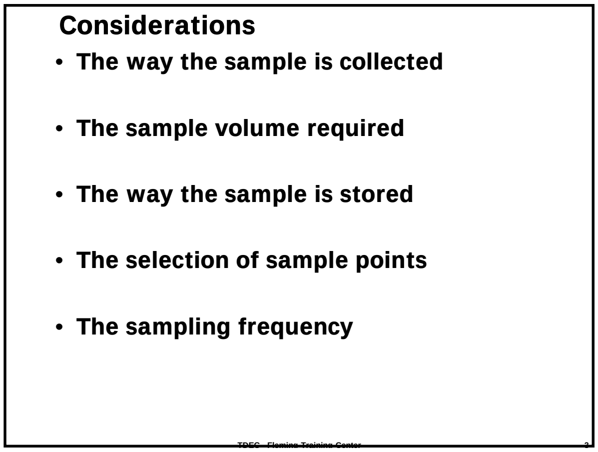
SAMPLING



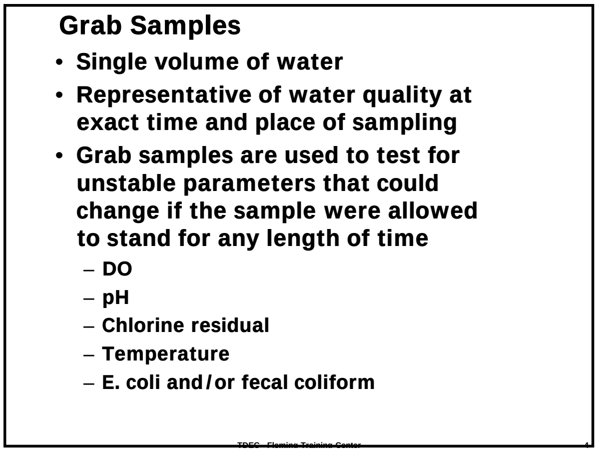
Why Sample?

- Meet compliance requirements
- Process control
- Ensure public safety / protect the environment



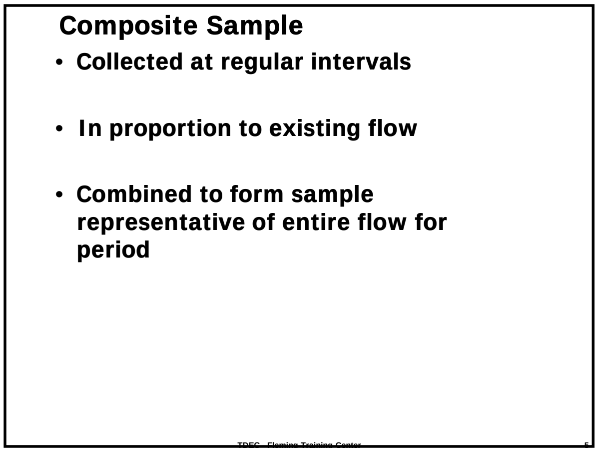
Considerations

- The way the sample is collected
- The sample volume required
- The way the sample is stored
- The selection of sample points
- The sampling frequency



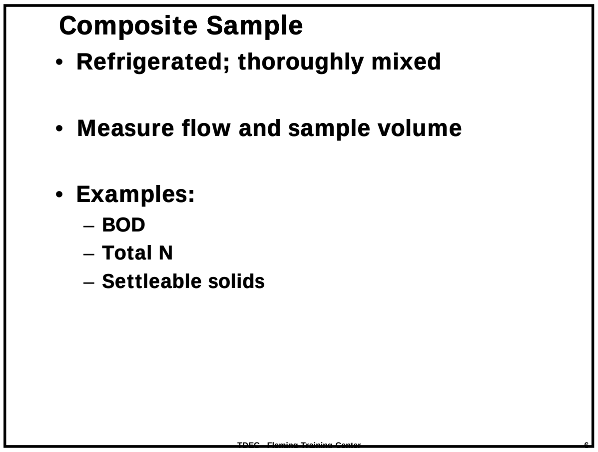
Grab Samples

- Single volume of water
- Representative of water quality at exact time and place of sampling
- Grab samples are used to test for unstable parameters that could change if the sample were allowed to stand for any length of time
  - DO
  - pH
  - Chlorine residual
  - Temperature
  - E. coli and / or fecal coliform



Composite Sample

- Collected at regular intervals
- In proportion to existing flow
- Combined to form sample representative of entire flow for period



Composite Sample

- Refrigerated; thoroughly mixed
- Measure flow and sample volume
- Examples:
  - BOD
  - Total N
  - Settleable solids

Sampling Guidelines

- Representative
- Proper container
- Do not contaminate the lid
- Preservative / dechlorinating agent

Sampling Guidelines

- Hold by base
- Turn into current
- Avoid air bubbles
- Label containers with sampler name; date and time; method; test to run; preservatives.

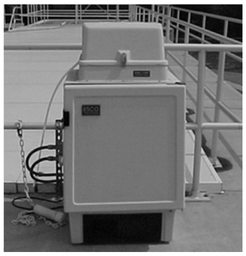
Sample Volume

- Depends on test procedure
- Headspace for mixing
- Preservative
- QA / QC comparisons

Sampling Point Selection

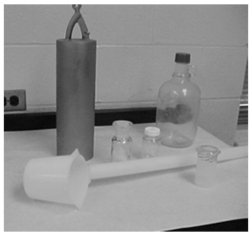
- Flow well mixed
- Exclude large particles (>1/4 inch)
- Exclude floating matter
- Readily accessible & in safe area

Sampling Devices

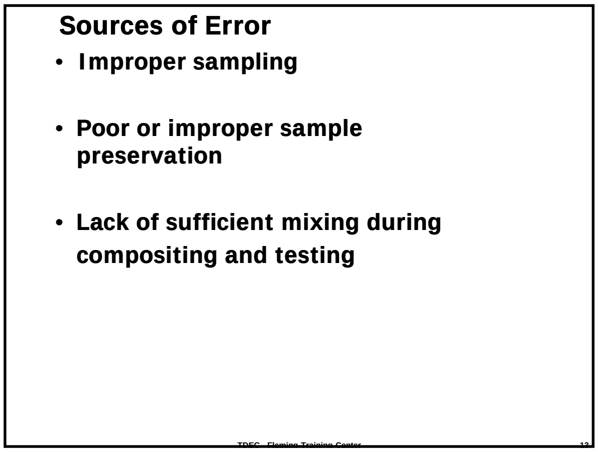


- Automatic:
  - Timesavers
  - Clean intake line regularly to prevent growth of bacteria

Sampling Devices

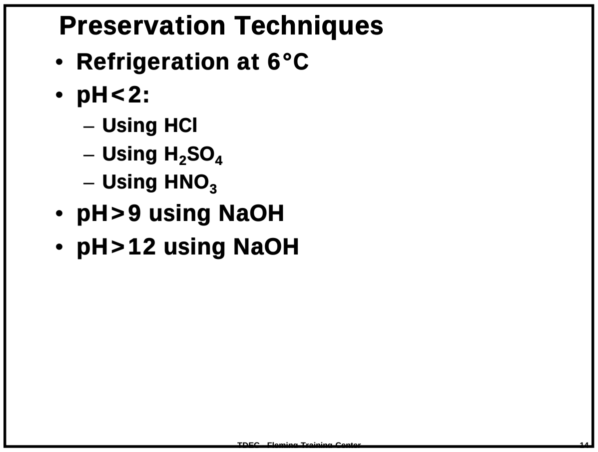


- Manual:
  - Dippers
  - Weighted bottle sampler
  - Whirl-pak® bags
  - Jugs



Sources of Error

- **Improper sampling**
- **Poor or improper sample preservation**
- **Lack of sufficient mixing during compositing and testing**



Preservation Techniques

- **Refrigeration at 6°C**
- **pH<2:**
  - **Using HCl**
  - **Using H_2SO_4**
  - **Using HNO_3**
- **pH>9 using NaOH**
- **pH>12 using NaOH**

Section 2
Dissolved Oxygen

Dissolved Oxygen and Temperature

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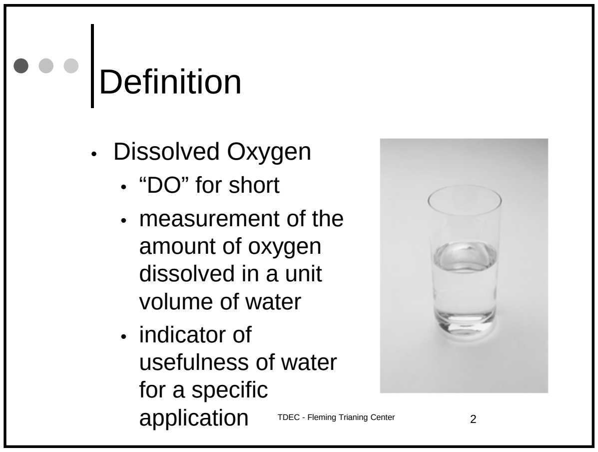
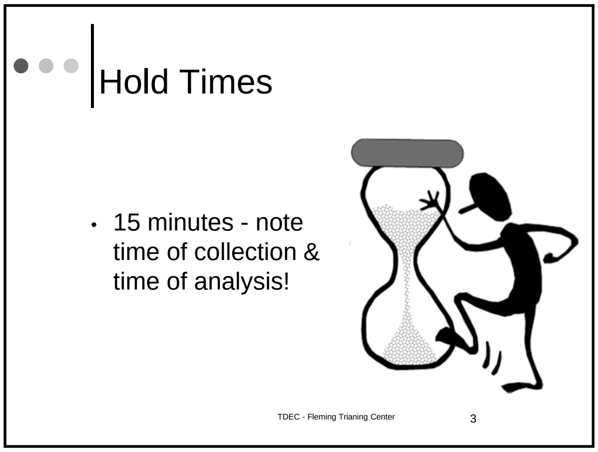


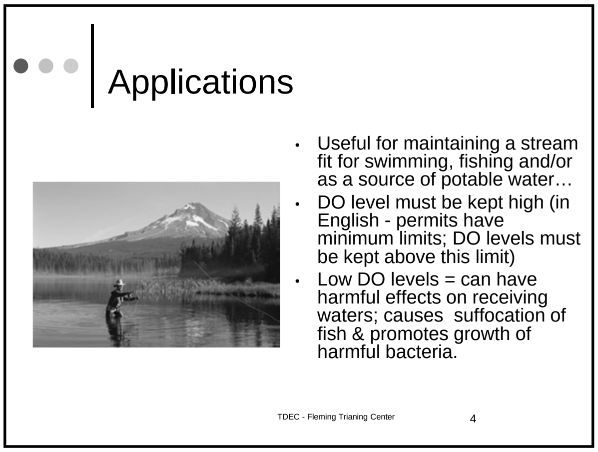
Definition

- Dissolved Oxygen
  - "DO" for short
  - measurement of the amount of oxygen dissolved in a unit volume of water
  - indicator of usefulness of water for a specific application



Hold Times

- 15 minutes - note time of collection & time of analysis!



Applications

- Useful for maintaining a stream fit for swimming, fishing and/or as a source of potable water…
- DO level must be kept high (in English - permits have minimum limits; DO levels must be kept above this limit)
- Low DO levels = can have harmful effects on receiving waters; causes suffocation of fish & promotes growth of harmful bacteria.

Applications

- Sewage treatment:
- Specialized bacteria (also known as activated sludge) is added to solids in waste water treatment plants
- optimum level of DO necessary for process
- too low DO = bacteria die & decompose
- too high DO = process becomes costly

Theory

- Amount of Oxygen that a given volume of water can hold is a function of:

1. The pressure the atmospheric oxygen is exerting at the air-water interface….
2. The temperature of the water.
3. The amount of other substances dissolved in the water.

Effect of Partial Pressure of Oxygen on Dissolved O_2

- Water in contact with air will absorb air (O_2)
  - until the pressure at air-water interface is equal…
  - said to be saturated - about 5 to 10 parts of oxygen to one million parts of water

Effect of Temperature on Dissolved Oxygen

- Pot of boiling water…
  - bubbles form on bottom & sides of pot…
  - number & size of bubbles increase with temperature….
  - These are air bubbles that have been dissolved in water.

Two Types of Measurement

- Electrode
- Winkler titration

Electrode

- An electrode system where DO reacts at the cathode producing a measurable electrochemical effect.
- Effect can be galvanic (ability to conduct an electrical current), polarographic (electrochemical), or potentiometric (measurement of voltages).

Electrodes

- Electrode system separated from the sample stream by semi-permeable membrane, which permits DO in sample to pass through to the electrode system, but prevents passage of liquids & ionic fluids.
- Most units are temperature compensated (thermistor or resistance thermometer).
- Another type of probe does not use semi-permeable membrane; system consists of a reference electrode & thallium measuring electrode.

Electrodes

- O_2 concentration determined by measuring voltage potential when DO comes in contact with the thallium electrode.
- Thallous-ion concentration is proportional to the dissolved oxygen in the sample.

Dissolved Oxygen

- Electrochemical method.
- Two metal electrodes in contact with electrolyte and separated from test solution by gas permeable membrane and a constant voltage created.
- Oxygen diffuses through membrane and is reduced at the cathode by the voltage.
- This process produces a current flow, which is detected by the meter, and is proportional to the partial pressure of oxygen.

Dissolved Oxygen

- Meter calibrated in saturated air
- Winkler test may be used as a QC check
- Correction for altitude or barometric pressure
- Membrane checked and changed regularly
- Temperature calibration data verified

Dissolved Oxygen Analysis

- Analyze immediately
  maximum holding time = fifteen min
- Preservation = none
- Sample should be measured insitu
- May be collected as a grab sample with minimal aeration into a B0D bottle with a glass stopper

Taking D.O. Readings

Common Deficiencies

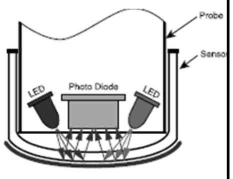
- Samples for dissolved oxygen were collected in a bucket and then poured into the BOD bottle
- The D.O. probe was immersed in the water during calibration
- There was an air bubble under the membrane on the probe
- The meter was air calibrated by placing the probe on the counter

Luminescent Dissolved Oxygen

- No membrane
- No electrolyte to foul or poison
- Won't affect readings
- Accurate & stable readings

Luminescent Dissolved Oxygen

- The sensor is coated with a luminescent material, called luminophore, which is excited by blue light from an internal LED.



Luminescent Dissolved Oxygen

- As the luminescent material relaxes it emits red light, and this luminescence is proportional to the dissolved oxygen present.
- The luminescence is measured both in terms of its maximum intensity and its decay time.
- An internal red LED provides a reference measurement before every reading to ensure that the sensor's accuracy is maintained.

Winkler Titration

- Winkler method (azide modification)
  - SM 4500-OC
  - Na_2SO_4 is to be standardized quarterly
  - Winkler test must be run within 8 hours of adding the reagents*, due to instability of solution once reagents are added. (*$MgSO_4$ & alkaline-iodine-azide solution)

Winkler

- Titrimetric wet chemistry test that measures the amount of oxygen present based on conversion of oxygen to iodine

Winkler

- Titrate with 0.025M Sodium Thiosulfate
  - Use a burette with 0.5 mL increments
- Titrate until a pale straw color

Temperature

- NIST (National Institute of Standards & Technology) traceable thermometer
- Scale marked in 0.1˚C
- Calibrate annually by checking against a NIST certified thermometer
- Corrections can be made up to ± 4˚C

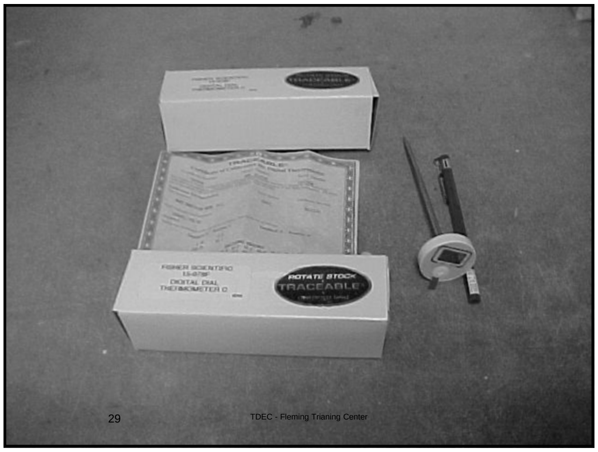
Temperature

- Clean the probe end with deionized water
- Swirl the thermometer in the sample and allow at least one minute to equilibrate
- Suspend the thermometer away from the sides and bottom of container
- Record readings to nearest 0.5˚C
- May be measured insitu with probe if verified against NIST traceable thermometer

Temperature Conversion

$$^\circ F = (9/5\ ^\circ C) + 32$$

$$^\circ C = 5/9\ (^\circ F - 32)$$



Oxygen, Dissolved

DOC316.53.01243

Direct Measurement Method **Method 10360**

(0.1 to 20.0 mg/L or 1 to 200% saturation) **LDO Probe**

Scope and Application: For water, wastewater and process water applications

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Meter	Probe
HQd meters	LDO101

Before starting the test:

Before attaching probes to the HQd meter for the first time, set the meter time and date.
Refer to the probe instructions for probe preparation.
For probes that are continuously immersed in aqueous solutions, condition the sensor cap for 72 hours.
When an IntelliCAL™ probe is connected to a HQ30d or HQ40d meter, the meter automatically recognizes the measurement parameter and is ready for use.
The IntelliCAL LDO101 probes automatically compensate for barometric pressure, elevation and temperature.
The LDO probe is calibrated at the factory. For more accurate results, manual calibration is recommended. Refer to the *Calibration* section of this procedure.
Salinity affects the concentration of dissolved oxygen in the sample. To correct for salinity effects, refer to Modifying LDO Measurement Options in the meter manual.

Collect the following items:

Description	Quantity
HQd meter	1
IntelliCAL LDO101 probe	1
Sensor cap for HQd with I-button	1
Shroud	1
BOD bottle, 300-mL or Erlenmeyer flask, 250-mL	1
Beaker, polypropylene (100-, 250-, 400- or 600-mL)	1

See *Consumables and replacement items* for reorder information.

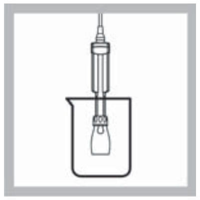
Method Name for powder pillows

1. Prepare the probe. Refer to the probe instructions.

2. Connect the probe to the meter.

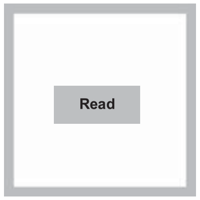
3. Calibrate the probe. Refer to the *Calibration* section of this procedure.

Go to step 4 for laboratory tests. Go to step 5 for field tests.



4. **Laboratory tests:** Immerse the probe in the beaker containing the sample solution. Move the probe up and down and tap it on the beaker to remove bubbles from the probe.

5. **Field tests:** Immerse the probe directly into the sample. Move the probe up and down to remove bubbles from the probe.



6. Press **READ** to store data in the data log.

Calibration

The LDO probe is calibrated at the factory. For more accurate results, manual calibration is recommended.

1. Remove the shroud from the probe body.
2. Add a small amount of water (about 1 cm) to the bottom of narrow-neck bottle, such as a BOD bottle.

   Note: *Use a wider neck bottle or flask (for example, a 250-mL Erlenmeyer flask) for the rugged probe.*
3. Insert a stopper and shake vigorously for several minutes.
4. Remove the stopper. If the sensor cap surface is wet, carefully dry the cap with a nonabrasive cloth, then put the probe in the bottle. Allow several minutes for the probe to reach equilibrium.

5. Make sure the meter is in the measurement screen. Press the **CALIBRATION** key.

   Note: *For HQ40d meters with two probes attached, the display must be in the single screen LDO101 mode.*

6. Press **READ**. When the measurement is stable, the calibrated measurement will show on the display. The standard value will be highlighted on the display.
7. Press **DONE** to view the calibration summary. The slope value is the comparison between the latest calibration and the factory calibration expressed as a percentage.

   Note: *If the calibration slope does not meet the acceptance criteria, the display will show "Slope out of range". Let the probe stand in water-saturated air for several minutes. When the probe reaches equilibrium, press* ***READ****.*

8. Press **STORE** to accept the calibration and return to the measurement mode. The calibration record is stored in the data log.

Note: *A successful calibration will show "OK" in the measurement screen.*

Interferences

There are no significant interferences with the LDO technology.

The IntelliCAL LDO101 probes are designed for water and wastewater applications, but can be used for other applications. Some organic solvents may damage the sensor cap and probe body.

Sample collection, preservation and storage

- Analyze samples in-situ, if possible.
- Collect samples in an appropriate container. Fill completely and analyze immediately.
- Do not store samples.

Accuracy check

1. Return the electrode to a water-saturated air environment.
2. Allow at least 10 minutes for stabilization.
3. Read the % saturation on the right side of the measurement mode screen. The meter should display 100% saturation. If not, allow additional time for the air to reach water saturation or calibrate the probe.

Method performance

The following statements are true for dissolved oxygen when the temperature is kept between 10 and 30 degrees C.

Method	Standard	Precision 95% Confidence Limits of Distribution	Accuracy Concentration change per 0.010 Abs change
10360	8.00 mg/L DO	7.95–8.05 mg/L DO	7.90–8.10 mg/L DO
10360	15.00 mg/L DO	14.90–15.10 mg/L DO	14.80–15.20 mg/L DO

Summary of method

The oxygen sensor is made up of a clear, oxygen impermeable hard substrate. An oxygen sensitive luminescent dye, along with a scattering agent, is pad-printed on the substrate. A final overlay of dark pigment is added to prevent stray light from entering the measurement cell. The luminescent dye emits red light when exposed to blue light. The scattering agent distributes the emitted light throughout the sensor matrix and contributes to the opacity of the sensor. Pulses from a red LED serve as an internal reference. The duration of the luminescence is proportional to the concentration of dissolved oxygen in the sample.

Consumables and replacement items

Required apparatus (select one)

Description	Quantity/Test	Unit	Catalog number
HQ40d multi-parameter meter, dual input	1	each	HQ40d53000000
HQ30d multi-parameter meter, single input	1	each	HQ30d53000000

Required probes (select one)

Description	Unit	Catalog number
LDO Probe, standard, with 1 m cable	each	LDO10101
LDO Probe, standard, with 3 m cable	each	LDO10103
LDO Probe, rugged, with 5 m cable	each	LDO10105
LDO Probe, rugged, with 10 m cable	each	LDO10110
LDO Probe, rugged, with 15 m cable	each	LDO10115
LDO Probe, rugged, with 30 m cable	each	LDO10130

Optional apparatus

Description	Unit	Catalog number
AC Power Adapter for HQd meters (included w/ HQ40d)	each	5826300
BOD bottle, 300 mL	each	62100
BOD bottle, 300 mL	6/pkg	62106
Citizen PD-24 USB Handy printer, 115 VAC	each	5835800
Color Coded Probe Clips (5 color coded sets) 5 sets	10/pkg	5818400
Depth Markers for Rugged LDO probe only	10/pkg	5828610
Erlenmeyer flask, 250 mL	each	2089846
Field Kit (Includes glove kit, 2 probe holders and 5 120 mL sample cups)[1]	each	5825800
Glove kit only for HQd meters	each	5828700
Probe Holder for HQd meter, IntelliCAL Standard probes only	each	5829400
Replacement Sensor cap w/ I-button	each	5811200
Replacement Shroud kit Rugged LDO probe	each	5825900
USB Keyboard for HQd meters (must have 5813400 & 5826300)	each	LZV582
USB/DC Adapter for HQd meters (must have 5826300, inc w/HQ40d)	each	5813400

[1] Included with HQ40d

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Updated February 2008, Edition 5

Section 3

Biochemical Oxygen Demand

TENNESSEE DEPARTMENT OF ENVIRONMENT AND CONSERVATION

BIOCHEMICAL OXYGEN DEMAND

BOD

OUTLINE

- Introduction to BOD
  - What is BOD?
  - Why is BOD Important?
  - BOD Calculations

OUTLINE

- Laboratory
  - Set up BOD Bottles
  - Measure Initial DO
- How is BOD Run?

WHAT IS BOD?

- Biochemical Oxygen Demand is the amount of oxygen, expressed in mg/L or parts per million (ppm), that bacteria take from the water when they oxidize organic matter.

(Hach, Clifford; R. Klein; C. Gibbs. Introduction to Biochemical Oxygen Demand. Hach Company, 1997.)

WHAT IS BOD?

- BOD is a measure of organic strength of the wastewater:
  - Changes in dissolved oxygen concentration are used as an indirect measure of organic content.

HISTORY OF BOD

- The Royal Commission on River Pollution, which was established in 1865 and the formation of the Royal Commission on Sewage Disposal in 1898 led to the selection in 1908 of BOD_5 as the definitive test for organic pollution of rivers.
- Five days was chosen as an appropriate test period because this is supposedly the longest time that river water takes to travel from source to the ocean in the U.K.

Source: http://en.wikipedia.org/wiki/Biochemical_oxygen_demand

History of BOD

- In 1912, the commission also set a standard of 20 mg/L BOD_5 as the maximum concentration permitted in sewage works discharging to rivers, provided that there was at least an 8:1 dilution available at dry weather flow.
- This was contained in the famous 20:30 (BOD:Suspended Solids) + full nitrification standard that was used as a yardstick in the U.K. up to the 1970s for sewage works effluent quality.

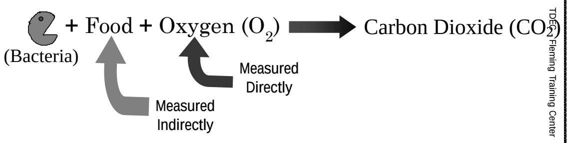
Source: http://en.wikipedia.org/wiki/Biochemical_oxygen_demand

History of BOD

- The United States includes BOD effluent limitations in its secondary treatment regulations.
- Secondary sewage treatment is generally expected to remove 85 percent of the BOD measured in sewage and produce effluent BOD concentrations with a 30-day average of less than 30 mg/L and a 7-day average of less than 45 mg/L.

Source: http://en.wikipedia.org/wiki/Biochemical_oxygen_demand

What is BOD?



(Bacteria) + Food + Oxygen (O_2) $\rightarrow$ Carbon Dioxide (CO_2)

What is BOD?

- All three must be present in order to have BOD.

, Food, and O_2

What is BOD?

(Bacteria) + Food + Oxygen (O_2) $\rightarrow$ Carbon Dioxide (CO_2)

Food - Organic material (carbon), exerts carbonaceous oxygen demand (cBOD)

What is BOD?

(Bacteria) + Ammonia or Nitrite (NO_2^-) + Oxygen (O_2) $\rightarrow$ Nitrate(NO_3^-)

Food – Reduced forms of nitrogen, exert nitrogenous oxygen demand

WHAT IS BOD?

Carbonaceous Oxygen Demand (cBOD) + Nitrogenous Oxygen Demand = BOD

WHAT IS BOD?

- Dissolved oxygen levels are monitored before and after an incubation period, to determine the amount of oxygen depletion.

WHAT IS BOD?

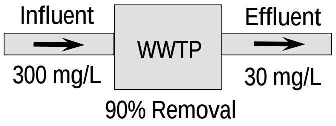
- Incubation conditions:
  - Temperature – 20 ± 1°C
  - Time – 5 days
  - In the dark

WHY IS BOD IMPORTANT?

- Wastewater treatment plants (WWTP) aim to remove BOD from the influent (raw) water.
  - BOD can be used for process control and to measure the efficiency of a WWTP.

WHY IS BOD IMPORTANT?

- BOD Removal Efficiency
  - BOD of effluent versus influent



Influent → WWTP → Effluent

300 mg/L

30 mg/L

90% Removal

WHY IS BOD IMPORTANT?

- BOD measurements help in monitoring the effect of effluent on the dissolved oxygen concentration of the receiving water body.
- BOD may be regulated by permit requirements.

BOD TEST REQUIREMENTS

- Multiple sample dilutions
- Dilution water blank
- Seed control
- Seed blank
- Glucose/Glutamic Acid Standard
- Duplicates

SAMPLE VOLUME DILUTION ESTIMATION

- Industrial wastes = 0.1 - 1.0 %
- Raw\settled sewage = 1.0 - 5.0 %
- Oxidized effluent = 5.0 - 25 %
- Polluted river water = 25 - 100 %

HOW IS BOD MEASURED?

Dilution Method

BODTrak

(Not EPA Approved for Permit Compliance)

BOD CALCULATIONS

- Using the dilution method, three values must be known in order to calculate BOD:
  - Initial DO
  - Final DO
  - Volume of sample

BOD DILUTION METHOD

BOD – DILUTION METHOD

- Glassware
- Preparing dilution water
- Seeding dilution water
- Sampling and sample handling
- Determining range and sample volumes
- Obtaining data points
- Running Standards

GLASSWARE

- Glassware must be extremely clean!
  - Have a set of glassware dedicated to BOD testing.
  - Clean glassware thoroughly before each use
    - Clean with a dilute bleach solution
    - Rinse at least 3x with DI water
    - Clean with 1:1 sulfuric acid
    - Rinse at least 3x with DI water
    - Allow to air dry

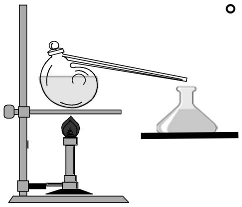
DILUTION WATER

- Source water
- Reagents
- Method criteria
- Failure factors
- Other considerations

DILUTION WATER SOURCE WATER

- 21st Ed. Standard Methods 5210B 4.C
  - Serves to check the quality of unseeded dilution water and the cleanliness of bottles
  - May be distilled, tap or receiving stream
    - As long as they meet depletion of < 0.2 mg/L
  - Free of heavy metals
    - Ex: copper

DILUTION WATER SOURCE WATER



- Preparing Dilution Water
  - Low-organic water can be prepared by distillation from alkaline permanganate.
    - Automatic commercial stills are available
    - Deionized or demineralized water is not recommended

DILUTION WATER REAGENTS

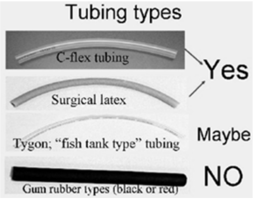
- Use clean glassware, tubing (medical grade) and bottles
- Distilled water should be used in preparing reagents in advance (sterilized is preferred)
- Discard if any sign of precipitation or biological growth
- Commercial reagents are acceptable

DILUTION WATER METHOD CRITERIA

- Preferably run two blanks, beginning and ending of sample set
- Dilution water blanks must meet quality control limits, < 0.2 mg/L DO (preferably <0.1 mg/L)
  - Otherwise discard and prepare fresh solution
- No seed or nitrification inhibitor is added for dilution water blank
- Run one nitrification inhibitor blank
- Total of three blanks
  - One dilution water blank at beginning
  - One nitrification blank at beginning
  - One dilution water blank at end

DILUTION WATER FAILURE FACTORS

- Check out this site for more information: http://dnr.wi.gov/org/es/science/lc/OUTREACH/BODResource/Troubleshoot.html
- Tubing constructed of oxygen-demand leaching material (consider medical grades)



DILUTION WATER FAILURE FACTORS

- Slime growth in delivery tubing
- Poor water quality/improperly maintained system
- Poorly cleaned bottles or dilution water storage container
- Improperly calibrated probe

DILUTION WATER FAILURE FACTORS

- Poor quality air for aeration.
- Deionizer systems can leach organics and grow bacteria.
  - Must be cleaned on a regular basis.
  - Try reverse osmosis (RO)/polisher combination systems that will produce ASTM Type 1 water.

DILUTION WATER OTHER CONSIDERATIONS

- Store bought distilled water
  - What are the containers made of?
  - QA/QC of water? What's in it?
- If it ain't broke, don't fix it.
  - If the water that you purchased works satisfactorily and shows good QA/QC, continue using it.
- Use an all glass still, avoid metal stills.

PREPARING DILUTION WATER

- Store distilled water in a BOD incubator until the temperature reaches 20^{o}C.
- Water is then saturated with oxygen and is ready for use.

PREPARING DILUTION WATER

Add buffer and nutrients

- After adding buffer and nutrients, shake the bottle vigorously to ensure saturation.
- Use immediately!
  - Storage of prepared dilution water for more than 24 hours after adding nutrients, minerals and buffer is not recommended unless dilution water blanks consistently meet quality control limits

Seeding Dilution Water

- Add seed to the dilution water (if needed)
- Seed can be added to:
  - Bulk dilution water preparation (good)
  - Individual bottles (better)

Seeding Dilution Water

- Seed may be necessary when testing:
  - Oxidized effluents
  - Toxic effluents
  - Samples with insufficient microorganisms
  - ***cBOD samples***

Seeding Dilution Water

- Sources of seed:
  - Settled domestic wastewater
  - Effluent from primary clarifiers
  - Diluted mixed liquor from an aeration basin
  - Undisinfected effluent
  - Receiving water from below the point of discharge
  - Purchased BOD seed
- When effluent of mixed liquor from a biological treatment process is used as a seed source, inhibition of nitrification is recommended

Seeding Dilution Water

- How much seed should be added?
  - Seed should contribute an oxygen demand ranging from 0.6-1.0 mg/L.
  - The amount of seed added should be adjusted from this range to that required to provide glucose-glutamic acid (GGA) check results of 198 ± 30.5 mg/L
  - For example, if 1 mL of seed is needed to achieve 198 ± 30.5 mg/L, then use 1 mL in each BOD bottle receiving the test wastewater

Seeding Dilution Water

- How is BOD of the seed determined?
  - Run seed control to determine the BOD of the seed.
  - Seed controls are run as if they were samples
    - Bottles containing dilution water and specific volumes of seed

Seed Control Factor

- Divide the DO depletion by the volume of seed in mL for each seed control bottle having a 2.0 mg/L depletion and greater than 1.0 mg/L minimum residual DO and average the results.

Sampling and Sample Handling

- Sampling Considerations
  - Preferable to sample BEFORE any disinfection
  - If sampling after any disinfection, samples MUST be seeded

Sampling and Sample Handling

- Sample Pre-Treatment
  - Composite samples kept at 1-10 °C
  - Recommended Hold Time =
    - Analysis must be run within 2 hours of collection (grab or end of 24-hour compositing period)
    - Refrigerate if unable to do so, ≤6° C
    - 48-hour holding time (40 CFR 136, Table II) @ ≤ 6°C
  - Sample Temperature (20 ± 1 °C)
  - Sample (Dilution) pH (6.5 - 7.5)
  - Check residual chlorine
    - If present, (1) quench chlorine, (2) seed samples
  - Samples Supersaturated? (DO > 9 mg/L at 20 °C)
    - Warm; shake or aerate to remove O2

Determining Range and Sample Volume

- Run a series of sample dilutions, at least 3 dilutions
  - Want 2 mg/L oxygen depletion in the first dilution
    - Minimum sample volume
  - Want 1 mg/L oxygen remaining in the last dilution
    - Maximum sample volume
  - Bring sample to 20 ± 3°C before checking initial DO

Determining Range and Sample Volume

- Fill bottles to top without adding bubbles
- If > 67% (200 mL) sample after dilution, add nutrients, mineral and buffer solutions directly to the sample at a rate of 1 mL/L (0.3 mL/300-mL bottle) or commercial prepared product
- Add NI to partially filled sample bottle for cBOD.
  - Seeding is required
- After preparing dilution, measure initial DO within 30 minutes

Determining Range and Sample Volume

First Dilution
2mg/L
oxygen
demand

Last Dilution
1mg/L
oxygen
remaining

Determining Range and Sample Volume

- Selection of sample volumes used in the test depends on two factors:
  - Type of sample
  - Elevation

DETERMINING RANGE AND SAMPLE VOLUME

Table 2 Determining Minimum Sample Volume

Sample Type	Estimated BOD mg/L	mL of Sample*
Strong Trade Waste	600	1
Raw and Settled Sewage	300	2
	200	3
	150	4
	120	5
	100	6
	75	8
	60	10
Oxidized Effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted River Waters	6	100
	4	200
	2	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

DETERMINING RANGE AND SAMPLE VOLUME

Table 3 Determining Maximum Sample Volume

Estimated BOD at			mL of Sample*
sea level	1000 ft.	5000 ft.	
2460	2380	2032	1
1230	1189	1016	2
820	793	677	3
615	595	508	4
492	476	406	5
410	397	339	6
304	294	251	8
246	238	203	10
205	198	169	12
164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

DETERMINING RANGE AND SAMPLE VOLUME

- If our sample is approximately 300mg/L BOD, what should the minimum and maximum sample volumes be?

DETERMINING RANGE AND SAMPLE VOLUME

Table 2 Determining Minimum Sample Volume

Sample Type	Estimated BOD mg/L	mL of Sample*
Strong Trade Waste	600	1
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	100	6
	75	8
	60	10
Oxidized Effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted River Waters	6	100
	4	200
	2	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

Table 3 Determining Maximum Sample Volume

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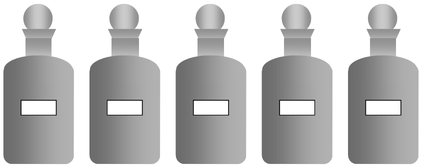
25	24	21	100
12	12	10	200
8	8	7	300

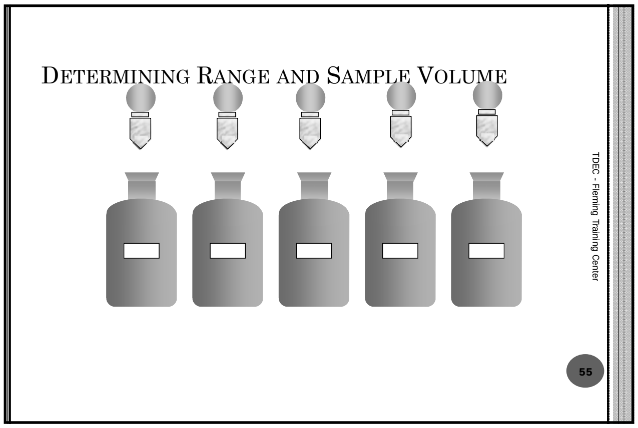
* mL of sample taken and diluted to 300 mL in standard BOD bottle

DETERMINING RANGE AND SAMPLE VOLUME

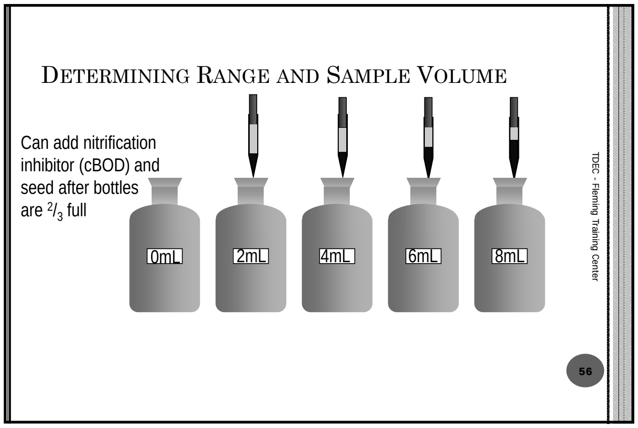
- If our sample is approximately 300mg/L BOD, what should the minimum and maximum sample volumes be?
  - Minimum volume = 2mL
  - Maximum volume = 8mL

DETERMINING RANGE AND SAMPLE VOLUME



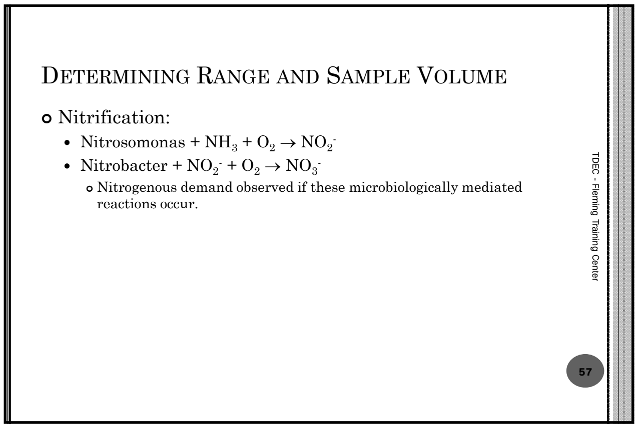


DETERMINING RANGE AND SAMPLE VOLUME



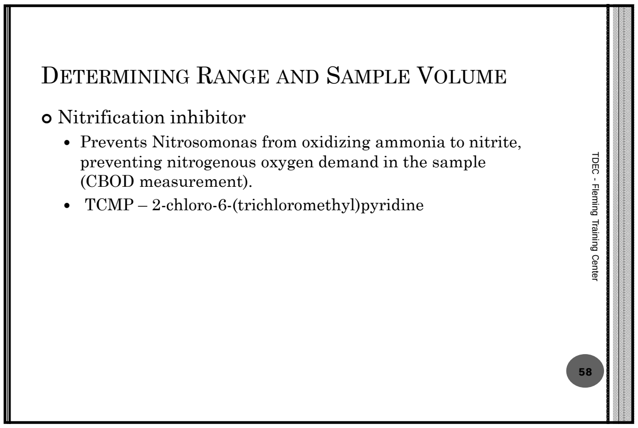
DETERMINING RANGE AND SAMPLE VOLUME

Can add nitrification inhibitor (cBOD) and seed after bottles are $^2/_3$ full



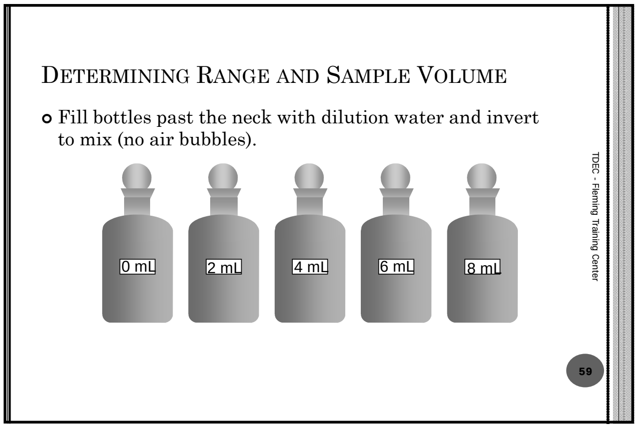
DETERMINING RANGE AND SAMPLE VOLUME

- Nitrification:
  - Nitrosomonas + $NH_3 + O_2 \rightarrow NO_2^-$
  - Nitrobacter + $NO_2^- + O_2 \rightarrow NO_3^-$
    - Nitrogenous demand observed if these microbiologically mediated reactions occur.



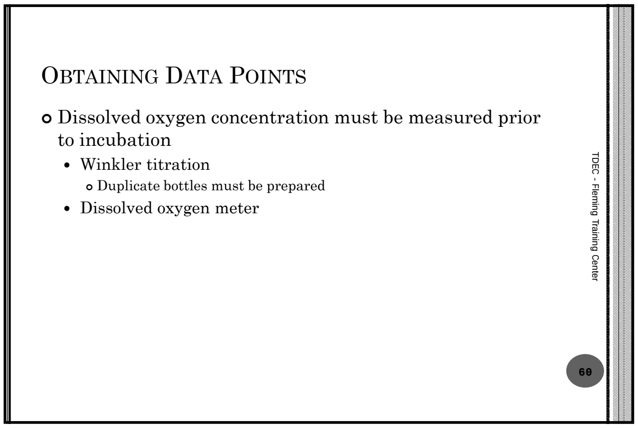
DETERMINING RANGE AND SAMPLE VOLUME

- Nitrification inhibitor
  - Prevents Nitrosomonas from oxidizing ammonia to nitrite, preventing nitrogenous oxygen demand in the sample (CBOD measurement).
  - TCMP – 2-chloro-6-(trichloromethyl)pyridine



DETERMINING RANGE AND SAMPLE VOLUME

- Fill bottles past the neck with dilution water and invert to mix (no air bubbles).



OBTAINING DATA POINTS

- Dissolved oxygen concentration must be measured prior to incubation
  - Winkler titration
    - Duplicate bottles must be prepared
  - Dissolved oxygen meter

OBTAINING DATA POINTS

- Measure DO
- Prior to measurement, prepare probe by:
  - Polarizing
  - Polishing
  - Calibrating
    - Water saturated air or versus Winkler titration

CALIBRATION

- Winkler titration - best; most accurate
  - Relies on chemistry
- Probe: Air-saturated water
  - Reagent water at 20°C shaken/aerated to saturate
  - Maximum DO at 20°C ~ 9.00 mg/L
  - Meter result shouldn't vary greatly from the saturation point
  - Correct for pressure and/or altitude differences

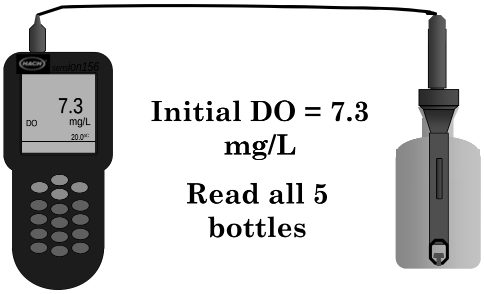
CALIBRATION

- Probe: Water-saturated air (most common)
  - Air-calibration chamber → calibrate at sample temperature.
  - Minimizes errors caused by temperature differences.
  - Keep interior of the chamber just moist -- not filled with water.
  - Typical for probes
  - Probe is stored in a constant humidity environment
  - Container should be sealed somehow (to maintain constant humidity)

OBTAINING DATA POINTS

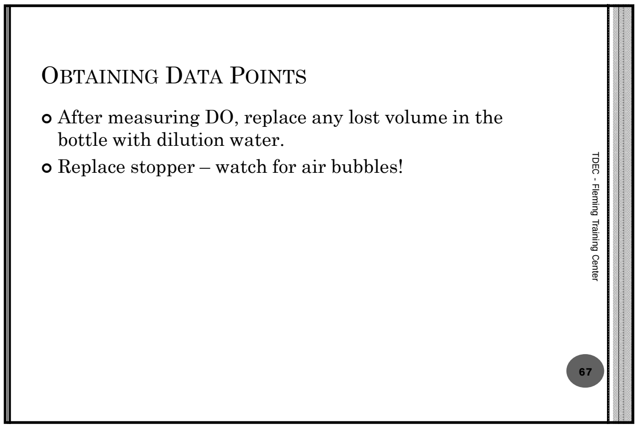
OBTAINING DATA POINTS

OBTAINING DATA POINTS



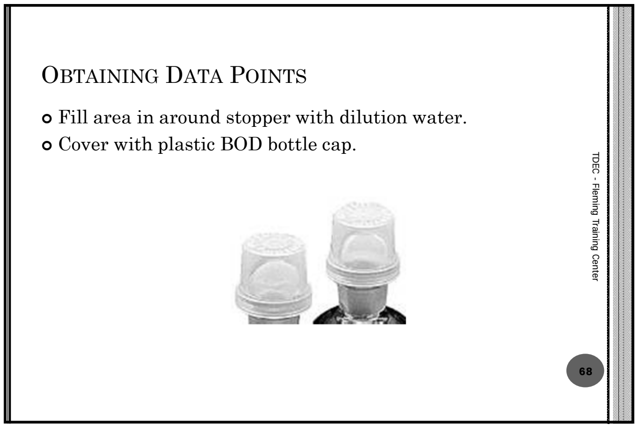
Initial DO = 7.3 mg/L

Read all 5 bottles



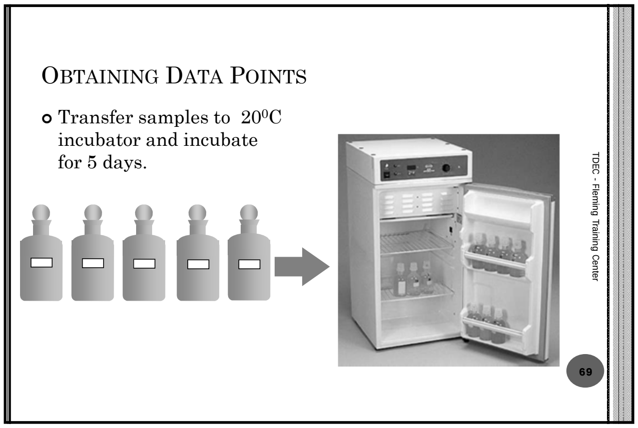
OBTAINING DATA POINTS

- After measuring DO, replace any lost volume in the bottle with dilution water.
- Replace stopper – watch for air bubbles!



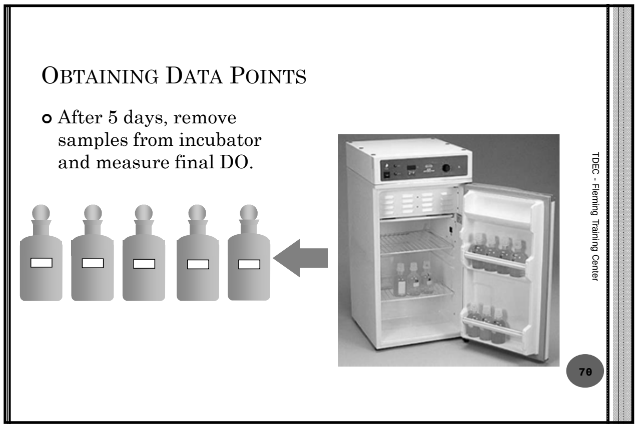
OBTAINING DATA POINTS

- Fill area in around stopper with dilution water.
- Cover with plastic BOD bottle cap.



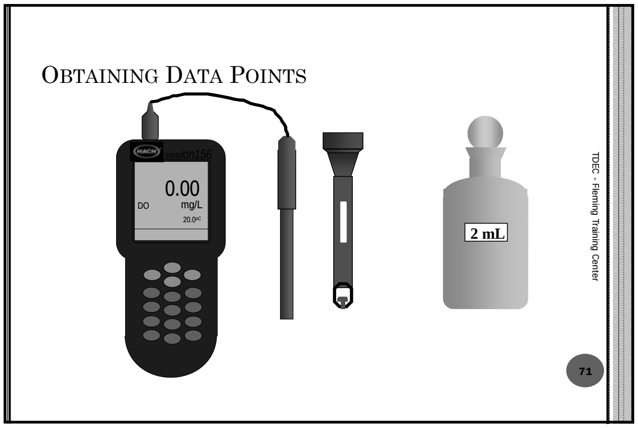
OBTAINING DATA POINTS

- Transfer samples to 20^0C incubator and incubate for 5 days.

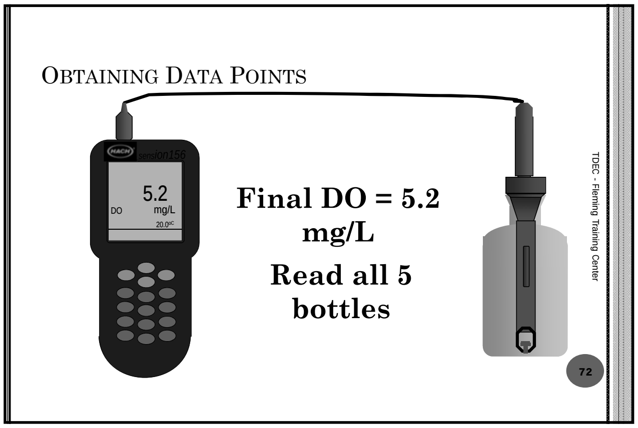


OBTAINING DATA POINTS

- After 5 days, remove samples from incubator and measure final DO.



OBTAINING DATA POINTS



OBTAINING DATA POINTS

Final DO = 5.2 mg/L

Read all 5 bottles

OBTAINING DATA

- Plug data into equation:

$$\text{BOD}_5\text{, mg/L} = \frac{(\text{Initial DO} - \text{Final DO})}{\text{P}}$$

OBTAINING DATA

- Plug data into equation:

$$\text{P} = 2/300 = 0.00667$$

$$\text{BOD}_5\text{, mg/L} = \frac{(7.3 - 5.2)}{0.00667}$$

$$\text{BOD}_5\text{, mg/L} = 315 \text{ mg/L}$$

OBTAINING DATA

- If bottles were seeded:

$$\text{BOD}_5\text{, mg/L} = \frac{(D_1 - D_2) - (B_1 - B_2)\,f}{\text{P}}$$

D = DO of sample

B = DO of seed

P = Sample volume/300

F = Ratio of seed in sample to seed in control

BOD SOLIDS HANDLING BIAS

- Homogeneous
- Use wide bore pipette
- Pipette as fast as possible to prevent loss of solids
- Pipette each sample dilution separately

BOD QC CHECKS

- Sample depletion criteria
- Seed control should also meet sample depletion criteria
  - Seed contribution should be between 0.6 and 1.O mg/L
  - Blank's DO depletion <0.2 mg/l
  - GGA range approx. 198 ± 30.5 mg/L

BOD QUALITY CONTROL

- Solubility of oxygen in water at 20^0C is 9.2 mg/L
- Super saturation = initial DO > 9.0 mg/L
- Average all dilutions that meet QC criteria
- Suspect toxicity if smallest sample concentration yields greatest uptake
- What if no samples meet criteria?

Common Sources Of Error

- Not adjusting pH to within 6.5 – 7.5
  - Adjustment not required if effluent is between 6.0-8.5
- Improper calibration of DO meter
- Incubation temperatures not constant
- Initial DOs above saturation

Common Sources Of Error

- Depletion criteria not met
- Subtracting blanks
- Not seeding when required
- Seed strength not constant
- Not analyzing GGA samples
- Not evaluating for toxicity
- Improper calculations
- Water quality issues

Running Standards

- Be careful purchasing GGA from Hach, ***some*** are not the same concentration as specified in Standard Methods
  - Standard Methods - 150 mg/L each GGA
  - Hach BOD Standard – 300mg/L each GGA (divide by 2)
  - Hach ezGGA – gives 198 mg/L BOD results

Running Standards

- The concentration of standard does NOT equal BOD.
  - 150mg/L each = 198 ± 30.5 mg/L BOD
  - 300mg/L each = 396 ± 61 mg/L BOD

BOD – Take Home Messages

- BOD is an indirect measure of organic content.
- BOD is measured by oxidizing organics using microorganisms (under specific conditions) and directly measuring the amount of oxygen consumed in the process.

Oxygen Demand, Biochemical

DOC316.53.01200

Dilution Method[1]

Method 8043

Scope and Application: For water and wastewater.

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater* and from Klein, R.L.; Gibbs, C. *Journal of Water Pollution Control Federation*, 1979, 51(9), 2257.

Test preparation

Before starting the test:

The BOD test is a 5-day test. Follow all steps carefully to make sure that the test does not have to be repeated.

The dilution water for this test must not have an oxygen demand or any toxins. When incubated for 5 days at 20 °C, the dissolved oxygen concentration in the dilution water must not change by more than 0.2 mg/L.

Carbonaceous BOD (CBOD) can be determined by the addition of nitrification inhibitor. A test for CBOD is recommended for biologically treated effluents, samples seeded with biologically treated effluents and river water.

The *Troubleshooting—Graphical calculation method* provides an alternate system for calculating results and is a convenient tool for troubleshooting problems in BOD measurements. The graphical calculation method is not approved for regulatory reporting.

Collect the following items:

Description	Quantity
BOD bottles, 300-mL, glass, with glass stoppers and plastic caps	6
Dilution water containing nutrient buffer and seed (see *Dilution water preparation*)	varies
Nitrification inhibitor (for CBOD only)	1 bottle
Pipet, serological	1
Incubator	1

See *Consumables and replacement items* for reorder information.

Dilution method

1. Prepare the dilution water using a BOD Nutrient Buffer Pillow. See *Dilution water preparation*.

2. Select the sample volumes. See *Sample size selection*.

***Note:** If the minimum sample volume is 3 mL or more, determine the dissolved oxygen in the undiluted sample; this determination can be omitted when analysing sewage and settled effluents known to have a dissolved oxygen content near 0 mg/L.*

When analyzing disinfected samples or industrial effluents, refer to *Interferences*.

3. Stir the sample gently with the pipet. Use the pipet to add the minimum sample volume to the first BOD bottle.

Add the remaining four sample volumes to four more BOD bottles. Mark the bottles and record the contents of each bottle.

4. Fill an additional BOD bottle with dilution water only. This will be the dilution water blank.

5. If the test is for CBOD, add two portions of Nitrification Inhibitor (approximately 0.16 g) to each bottle.

The oxidation of nitrogen compounds will be prevented. Report results as CBOD.

6. Fill each bottle to just below the lip with dilution water. Allow the dilution water to flow down the sides of the bottle to prevent air bubbles from becoming trapped in the bottle.

7. Stopper the bottles carefully to prevent air bubbles from becoming trapped. Tightly twist the stopper into place. Press down on the stopper and invert the bottles several times to mix.

8. Measure the initial dissolved oxygen concentration in each bottle. Use a probe and meter or titration. If a titration is used, two sets of BOD bottles must be prepared.

Be sure to measure the DO of the dilution water blank.

Dilution method

9. Stopper the bottles carefully to prevent air bubbles from becoming trapped. Add dilution water to the lip of each BOD bottle to make a water seal.

10. Place a plastic cap over the lip of each bottle. Put the bottles in an incubator at 20 (±1) °C. Incubate for five days.

11. After five days, measure the remaining dissolved oxygen concentration in each bottle.

At least 1.0 mg/L DO should be left in each BOD bottle.

12. Calculate the BOD value (see *Calculation Methods—Standard Methods*).

Dilution water preparation

The dilution water must be prepared very carefully to make sure that no source of oxygen demand or toxins are added. The water that is used to prepare the dilution water must be of very high quality. The water must not have any organic compounds or any toxic compounds such as chlorine, copper and mercury.

Use the following guidelines to make sure the dilution water is of high quality.

Guidelines

- Use distilled water from an alkaline permanganate distillation for the best results.
- Do not use deionized water from ion exchange columns. The resins in the cartridges (especially new cartridges) will occasionally release organic materials that have an oxygen demand. In addition, bacteria can grow on the columns and contaminate the dilution water.
- Store the distilled water in clean jugs in an incubator at 20 °C. Fill containers till about ¾ full and shake the jugs to saturate the water with air, or cap the jugs loosely and store for 24 hours or more, to allow dissolution of oxygen.
- A small aquarium pump or air compressor can be used to saturate the water with air. Make sure that the air is filtered and that the filter does not grow bacteria. Clean the apparatus before and after use.
- Add the nutrients and seed (if necessary) to the distilled water immediately before the test.
- The dissolved oxygen concentration in the dilution water must not change by more than 0.2 mg/L when incubated for 5 days at 20 °C.

Procedure

1. Prepare and store the distilled water at 20 °C (see *Guidelines*).
2. Select a BOD nutrient buffer pillow from the *BOD nutrient buffer pillows* table.
3. Tap the pillow on a hard surface then shake the pillow to mix the contents.

4. Add the contents of the pillow to the distilled water in a jug with ample headspace above the water. Cap the jug and shake vigorously for one minute to dissolve the nutrients and to saturate the water with air.
5. If the sample is known to be low in bacteria, for example industrial waste or sewage that has been disinfected, add 3 mL of bacterial seed to each liter of the dilution water. Use raw sewage for the bacterial seed. Allow the sewage to stand undisturbed at 20 °C for 24 to 36 hours before use. Pipet from the upper portion of the sewage. Make sure to measure the BOD of the seed so that it can be subtracted from the BOD of the sample. A seed that has a BOD of 200 mg/L (a typical range for domestic sewage) will typically deplete at least 0.6 mg/L DO, when added at a rate of 3 mL/L of dilution water. If insufficient oxygen depletion occurs, increase the quantity of the seed.

Table 1 BOD nutrient buffer pillows

Volume of dilution water to prepare	BOD nutrient buffer pillow catalog no.
300 mL (add pillow to each BOD bottle)	1416066
3 liters	1486166
4 liters	2436466
6 liters	1486266
19 liters	1486398

***Note:** To prepare dilution water by the conventional method, pipet 1 mL of each of the following solutions per liter of distilled water at 20 °C: Calcium Chloride Solution, Ferric Chloride Solution, Magnesium Sulfate Solution, and Phosphate Buffer Solution. Cap the bottle and shake vigorously for one minute. The Phosphate Buffer Solution should be refrigerated to decrease the rate of biological growth. Use care with all solutions to avoid contamination.*

Sample size selection

Make an estimation of the sample volumes that are necessary for the test. At least 2.0 mg/L of dissolved oxygen (DO) should be consumed during the test and at least 1.0 mg/L DO should be left in the BOD bottle.

Samples such as raw sewage will have a high BOD. Small sample volumes must be used because large samples will consume all of the oxygen. Samples with a low BOD must use larger sample volumes to make sure that enough oxygen is consumed to give accurate results.

The elevation of the laboratory changes the amount of oxygen that can dissolve in water (see *Oxygen saturation values at various altitudes (20 °C)*). At higher elevations, the amount of oxygen that can dissolve in water decreases, so less oxygen is available to microorganisms.

Procedure

1. Refer to the *Minimum sample volume* table to select the minimum sample volume. For example, if a sewage sample is estimated to contain 300 mg/L BOD, the minimum sample volume is 2 mL. For sewage effluent with an estimated BOD of 40 mg/L, the minimum sample volume is 15 mL.
2. Refer to the *Maximum sample volume* table to select the maximum sample volume. At 1000 feet, with an estimated BOD of 300 mg/L, the largest sample volume is 8 mL. For a BOD of 40 mg/L the maximum volume is 60 mL (also at 1000 feet).
3. Select two or more other sample volumes between the minimum and maximum volumes so that there are four or five sample volumes total.

Table 2 Minimum sample volume

Sample type	Estimated BOD (mg/L)	Minimum sample volume (mL)
Strong trade waste	600	1
Raw and settled sewage	300	2
	200	3
	150	4
	120	5
	100	6
	75	8
	60	10
Oxidized effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted river waters	6	100
	4	200
	2	300

Table 3 Maximum sample volume[1]

BOD at sea level	BOD at 1000 ft	BOD at 5000 ft	Maximum sample volume (mL)
615	595	508	4
492	476	406	5
410	397	339	6
304	294	251	8
246	238	203	10
205	198	169	12
164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

[1] Samples with higher concentrations should be pre-diluted, per Standard Methods.

Table 4 Oxygen saturation values at various altitudes (20 °C)

Altitude (ft)	Average Pressure in mBar at this altitude	Oxygen value (mg/L) in water saturated with air
Sea level (0)	1013	9.09
1000	977	8.76
2000	942	8.44
3000	908	8.13
4000	875	7.82
5000	843	7.53
6000	812	7.24

Calculation Methods—Standard Methods

Use the Standard Methods calculation when the results must be reported to a regulatory agency.

When dilution water is not seeded:

$$\text{BOD}_5,\ \text{mg/L} = \frac{D_1 - D_2}{P}$$

When dilution water is seeded:

$$\text{BOD}_5,\ \text{mg/L} = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

where:

BOD_5 = BOD value from the 5-day test

D^1 = DO of diluted sample immediately after preparation, in mg/L

D^2 = DO of diluted sample after 5 day incubation at 20 °C, in mg/L

P = Decimal volumetric fraction of sample used

B^1 = DO of seed control before incubation, in mg/L

B^2 = DO of seed control after incubation, in mg/L

f = ratio of seed in diluted sample to seed in seed control =

(% seed in diluted sample)/(% seed in seed control) OR

If seed material is added directly to sample or to seed control bottles:

f = (volume of seed in diluted sample)/(volume of seed in seed control)

Report results as $CBOD_5$ if nitrification inhibitor was added.

Averaged results are acceptable if more than one sample dilution meets all of the following criteria:

- The remaining DO is at least 1 mg/L
- The final DO value is at least 2 mg/L lower than the initial DO value
- There is no evidence of toxicity at higher sample concentrations
- There are no obvious anomalies

Interferences

Many chlorinated and industrial effluents require special handling to ensure reliable BOD results. Usually, careful experimentation with the particular sample will indicate what modifications should be made to the test procedure.

Toxins in the sample will adversely affect any microorganisms present and result in lower BODs.

To eliminate small amounts of residual chlorine, allow the sample to stand for one to two hours at room temperature. For larger quantities, determine the amount of sodium thiosulfate to add to the sample as follows:

- **a.** Measure 100 mL of sample into a 250-mL Erlenmeyer flask. Using a 10-mL serological pipet and a pipet filler, add 10 mL of 0.020 N Sulfuric Acid Standard Solution and 10 mL of Potassium Iodide Solution, 100-g/L, to the flask.
- **b.** Add three full droppers of Starch Indicator Solution and swirl to mix.
- **c.** Fill a 25-mL buret with 0.025 N Sodium Thiosulfate Standard Solution and titrate the sample from dark blue to colorless.
- **d.** Calculate the amount of 0.025 N Sodium Thiosulfate Standard Solution to add to the sample:

$$\text{mL 0.025 N sodium thiosulfate required} = \frac{\text{mL titrant used x volume of remaining sample}}{100}$$

- **e.** Add the required amount of 0.025 N Sodium Thiosulfate Standard Solution to the sample. Mix thoroughly. Wait 10 to 20 minutes before running the BOD test.

To eliminate the effect of phenols, heavy metals or cyanide, dilute the sample with high quality distilled water. Alternately, the seed used in the dilution water may be acclimatized to tolerate such materials. Acclimatize seed as follows:

- **a.** Fill a one-gallon stainless steel or plastic container with domestic sewage and aerate for 24 hours. Allow the heavier material to settle.
- **b.** After settling for one hour, siphon off three quarts of material and discard.
- **c.** Fill the container with a mixture of 90% sewage and 10% wastes containing the toxic material.
- **d.** Aerate for 24 hours. Repeat steps b and c with increasing amounts of waste until the container holds 100% toxic waste material.

Optimum pH for the BOD test is between 6.5 and 7.5. Adjust samples to pH 7.2 with Phosphate Buffer Solution or 1 N Sulfuric Acid or Sodium Hydroxide Standard Solution if the pH is not in this range.

Cold samples may be supersaturated with oxygen and will have low BOD results. Fill a one-quart bottle about halfway with cold sample and shake vigorously for two minutes. Allow sample to reach 20 °C. Then shake the bottle vigorously for two minutes.

Accuracy check

ezGGA Method
Required for accuracy check:

- BOD Standard Solution, Voluette® Ampule, 300-mg/L, 10-mL (300-mg/L of glucose and 300-mg/L of glutamic acid)
- Seeded dilution water
- 4 BOD bottles
- 1.0–4.0 mL Class A volumetric pipets and pipet filler or 1–10 mL TenSette Pipet and Pipet tips
- Dissolved oxygen measurement apparatus

DO measurement with the LBOD probe:

1. Add the necessary seed to a 300-mL BOD bottle.
2. Fill the BOD bottle with dilution water until the water level is approximately ¼ inch up the ground glass portion of the neck. (See dimension “x” in illustration).
3. Put the 2-mL BOD standard ampule into the ampule breaker and rinse the assembly with deionized water.
4. Hold the ampule and breaker over the rim of the BOD bottle.
5. Use the ampule breaker to open the ampule and allow it to fall into the BOD bottle. Leave ampule in the BOD bottle during incubation period.
6. Follow the general procedure for the BOD test.
7. Calculate the BOD concentration of the standard solution. The 2 mL in the vial is equivalent to 6 mL as prepared by Standard Methods. Calculate the BOD concentration as though there were 6 mL added to the bottle instead of 2 mL. The dilution factor for this standard is 50x.

DO measurement with the Clark Cell electrode:

1. Add the necessary seed to a 300-mL BOD bottle.
2. Use the ampule breaker to open the ampule.
3. Pour the contents of the ampule into the BOD bottle. Tap the ampule on the rim of the bottle to dislodge the contents. Do not drop ampule into the bottle when using a Clark Cell.
4. Fill the ampule with buffered dilution water and add the water to the BOD bottle.
5. Repeat step 4.
6. Fill the BOD bottle with dilution water until the water level is approximately ½ inch up the ground glass portion of the neck.
7. Follow the general procedure for the BOD test.
8. Calculate the BOD concentration of the standard solution. The 2 mL in the vial is equivalent to 6 mL as prepared by Standard Methods. Calculate the BOD concentration as though there were 6 mL added to the bottle instead of 2 mL. The dilution factor for this standard is 50x.

Note: *The ampules include precisely 2 mL of 450 mg/L GGA. Pouring the entire solution into the bottle is the same as adding 6 mL of 150 mg/L solution as per the Standard Methods.*

Troubleshooting—Graphical calculation method

The Graphical Method helps troubleshoot problems in BOD measurements. This method cannot be used when the results must be reported to a regulatory agency.

1. Plot the mg/L dissolved oxygen (DO) remaining in each diluted sample versus the mL sample taken. Draw the best straight line through the plotted points. See *Dissolved Oxygen per mL of Sample*.

Note: *An erroneous point is visually evident at this time and can be disregarded. However, at least three points should be on the line or very close to it. For unseeded dilution water, the line should cross the mg/L DO Remaining scale near or below the oxygen saturation value for the altitude of the laboratory as discussed in Dilution water preparation.*

2. To calculate the BOD, use the following equation which is mathematically equivalent to the BOD equation in Standard Methods.

mg/L BOD = (A x 300) – B + C

where:

A = the slope

The slope of the line is equal to the mg/L DO consumed per mL of sample taken. Take any point on the line and subtract the mg/L DO Remaining at that point from the mg/L DO where the line crosses the DO scale (Y intercept, mg/L DO Remaining). Divide the difference by the mL of sample at the point chosen.

300 = the volume of the BOD bottle

B = the Y intercept

This is the DO value where the line crosses the "DO Remaining" scale. (This should be very close to the actual dilution water blank value.)

C = the sample DO

This is the DO of the undiluted sample.

Another way to write this equation is:

mg/L BOD = (Slope x 300) – Y intercept + Sample DO

Note: *If the best straight line is obtained by linear regression through use of a calculator, the sign (-) of the slope must be changed (+) before multiplying by 300.*

Example:
The mg/L DO remaining was determined for a series of four dilutions of domestic sewage after five days of incubation. Results were as follows:

mL of sample taken	mg/L DO remaining
2.0	7.50
3.0	6.75
6.0	4.50
9.0	2.25

The DO values were plotted versus the mL of sample taken and a straight line drawn as in *Dissolved Oxygen per mL of Sample*. If a set of BOD dilutions is run correctly with a homogeneous sample, a graph of the mg/L DO remaining versus the sample volume would result in a straight line. The value where the line intersects the y-axis is equal to the DO content of the dilution water

after incubation, although this is not actually measured. In this case, it was equal to 9.0 mg/L and the DO of the domestic sewage sample was assumed to be zero. If another type of sample is used, the DO of an undiluted sample should be measured either by the Winkler titration or with a luminescent or electrochemical probe.

The *Calculation Methods—Standard Methods* formula for calculating BOD also can be written as follows (not approved for reporting purposes):

$$\frac{\text{mg/L DO remaining w/smaller sample volume} - \text{mg/L DO remaining w/larger sample volume}}{\text{mL of larger sample volume} - \text{mL of smaller sample volume}} \times 300 - DO_D + S = \text{mg/L BOD}$$

Using this information in the example:

mg/L DO remaining with smaller sample volume = 7.50

mg/L DO remaining with larger sample volume = 2.25

mL of larger sample volume = 9.0

mL of smaller sample volume = 2.0

300 = volume (mL) of BOD bottle

DO_D = mg/L DO of dilution water = 9.0

S = mg/L DO of sample = assumed in this case to be zero

Therefore:

$$\frac{7.50 - 2.25}{9.0 - 2.0} \times 300 - 9 + 0 = \text{mg/L BOD} = 216 \text{ mg/L BOD}$$

Using the equation below:

(slope x 300) – Y-Intercept + sample DO = mg/L BOD

To determine slope, arbitrarily select point A in Figure 1. At this point the mg/L DO remaining is equal to 3.0 mg/L. The mL of sample at this point is 8 mL.The difference between the y-intercept of 9.0 mg/L and 3.0 mg/L equals 6 mg/L; 6 mg/L divided by 8 mL = 0.75 mg/L per mL.

slope = 0.75 mg/L per mL

Y intercept = 9.0 mg/L

sample DO = 0 (Because the sample is domestic sewage, this is assumed to be zero.)

Therefore:

(0.75 x 300) – 9.0 + 0 = mg/L BOD = 216 mg/L BOD

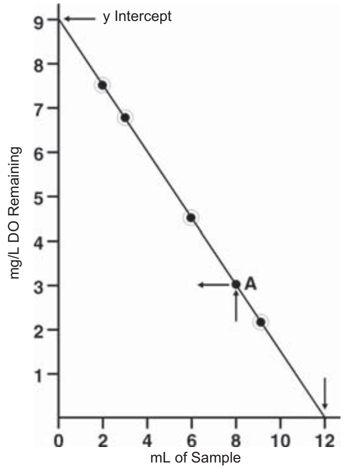


Figure 1 Dissolved Oxygen per mL of Sample

Summary of method

Biochemical Oxygen Demand (BOD) is a measurement of the oxygen requirements of municipal and industrial wastewaters and sewage. The test results are used to calculate the effect of waste discharges on the oxygen resources of the receiving waters. The BOD test is of limited value in measuring the actual oxygen demand because temperature change, biological population, water movement, sunlight, oxygen concentration, and other environmental factors cannot be reproduced accurately in the laboratory. The BOD test is of greatest value after patterns of oxygen uptake for a specific effluent and receiving water have been established.

The BOD test is performed by incubating a sealed wastewater sample (or a prepared dilution) for the standard five-day period and then determining the change in dissolved oxygen content. The BOD value is then calculated from the results of the dissolved oxygen tests.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
BOD Nutrient Buffer Pillows, for 3 liters of dilution water	1 pillow	50/pkg	1486166

Required apparatus

Description	Quantity/Test	Unit	Catalog number
BOD Bottle, glass-stoppered, 300-mL, unlabelled	6	6/pkg	62106
BOD Bottle Cap	6	6/pkg	241906
Bottle, wash, 500-mL	1	each	62011
Clippers, large	1	each	96800
Pipet, serological:			
Pipet, serological, 1-mL	1	each	919002
Pipet, serological, 5-mL	1	each	53237
Pipet, serological, 10-mL	1	each	53238
Pipet Filler	1	each	1218900
Dissolved Oxygen measurement apparatus	—	—	—

Recommended standards

Description	Unit	Catalog number
BOD Standard Solution, Voluette® Ampule, 300-mg/L, 10-mL	16/pkg	1486510
ezGGA BOD Standard Ampules, 450 mg/L, 2 mL	20/pkg	—

Product Lot # : ____________ **Technician:** ____________

Date In: ____________ **Date Out:** ____________

Temp In: ____________ **Temp Out:** ____________

Standard Method 5210B

1. BLANKS: Checks the BOD water & BOD bottles

DEP MUST BE <0.2 mgL

Bottle #	Initial DO	Final DO	Depletion
			0.00
			0.00
NI Blank			0.00
	AVERAGE BLANKS:		**0.00**

2. SEEDED CONTROLS (SCF): Calculates the effect of PolySeed Nx

f= ml of PS used in samples/GGA 4.0 Dep. Must be > 2 mg/L

Must be between: 0.6-1.0

Bottle #	Vol. (mL) of PolySeed	Initial DO B1	Final DO B2	Depletion B1 - B2	% Dep.	BOD	SCF (B1-B2)f
	5			0.0000	#DIV/0!	0.0	0.0000
	10			0.0000	#DIV/0!	0.0	0.0000
	15			0.0000	#DIV/0!	0.0	0.0000
					AVERAGE SCF:		**0.0000**

3. GGA STANDARD: Tests the seed against prepared STD

Calculation uses the average SCF from cell H21

Must be: 198 ± 30.5

Bottle #	Vol. (mL) of GGA	Vol. (mL) of PolySeed	Initial DO	Final DO > 1 mg/L	Depletion > 2 mg/L	Net. Dep	% Dep.	BOD
	6	4			0.0000	0.00	#DIV/0!	0.0
	6	4			0.0000	0.00	#DIV/0!	0.0
						AVERAGE GGA:		**0.0000**

4. SAMPLES: Calculates the BOD_5 of the Sample

Calculation uses the average SCF from cell H21

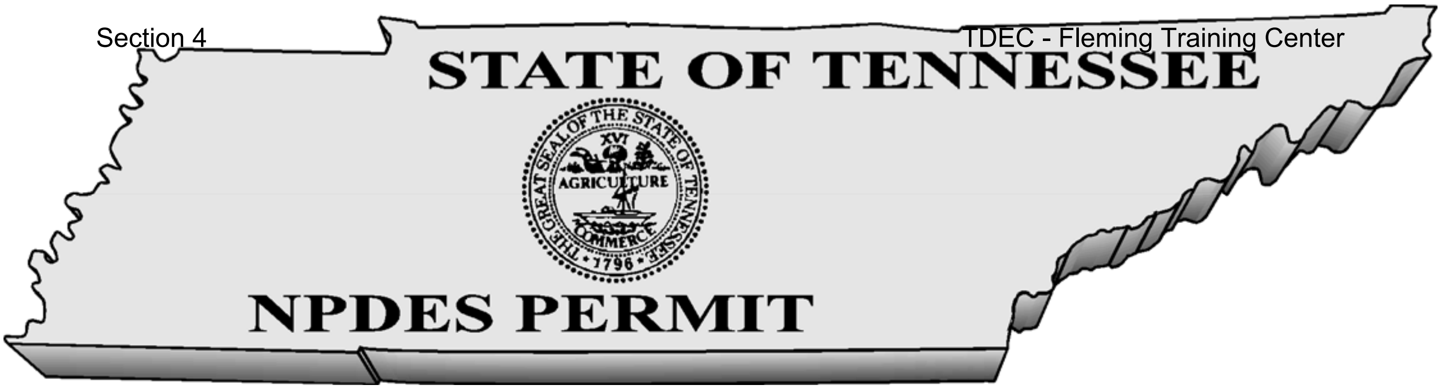
				>1 mg/L	> 2 mg/L	**Influent**			
Bottle #	Vol. (mL) of sample	Vol. (mL) of PolySeed	Initial DO D1	Final DO D2	Depletion D1-D2	SCF (B1-B2)f	Net Dep (D1-D2)-(B1-B2)f	Dil. Factor P	Sample BOD [(D1-D2)-(B1-B2)f]/P
	5	4			0.0000	0.0000	0.00	0.02	0.00
	10	4			0.0000	0.0000	0.00	0.03	0.00
	15	4			0.0000	0.0000	0.00	0.05	0.00
							AVERAGE $cBOD_5$:		**0.0000**

5. SAMPLES: Calculates the BOD_5 of the Sample

Calculation uses the average SCF from cell H21

				>1 mg/L	> 2 mg/L	**Effluent**			
Bottle #	Vol. (mL) of sample	Vol. (mL) of PolySeed	Initial DO D1	Final DO D2	Depletion D1-D2	SCF (B1-B2)f	Net Dep (D1-D2)-(B1-B2)f	Dil. Factor P	Sample BOD [(D1-D2)-(B1-B2)f]/P
	300	4			0.0000	0.0000	0.00	1.00	0.00
	275	4			0.0000	0.0000	0.00	0.92	0.00
	250	4			0.0000	0.0000	0.00	0.83	0.00
							AVERAGE $cBOD_5$:		**0.0000**

Section 4
Approved Methods and NPDES



STATE OF TENNESSEE

NPDES PERMIT

No. TN00-----

Authorization to discharge under the
National Pollutant Discharge Elimination System (NPDES)

Issued By

Tennessee Department of Environment and Conservation
Division of Water Pollution Control
401 Church Street
6th Floor, L & C Annex
Nashville, Tennessee 37243-1534

Under authority of the Tennessee Water Quality Control Act of 1977 (T.C.A. 69-3-101 et seq.) and the delegation of authority from the United States Environmental Protection Agency under the Federal Water Pollution Control Act, as amended by the Clean Water Act of 1977 (33 U.S.C. 1251, et seq.)

Discharger:	**--- STP**
is authorized to discharge:	**Treated municipal wastewater**
from a facility located:	**in ---, --- County, Tennessee**
to receiving waters named:	**Receiving stream Mile --**

in accordance with effluent limitations, monitoring requirements and other conditions set forth herein.

This permit shall become effective on:

This permit shall expire on:

Issuance date:

Paul E. Davis, Director
Division of Water Pollution Control

CN-0759
(Template Rev. 1-05)

RDAs 2352 and 2366

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MTS
TN00-----PMT.DOC

1.0. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

1.1. NUMERIC AND NARRATIVE EFFLUENT LIMITATIONS

The City of --- is authorized to discharge Treated municipal wastewater to the Receiving stream Mile --. Discharge 001 consists of municipal wastewater from a treatment facility with a design capacity of 2 MGD. Discharge 001 shall be limited and monitored by the permittee as specified below:

Effluent Characteristics	**Effluent Limitations**						**Monitoring Requirements**		
	Monthly Average Conc. (mg/l)	Monthly Average Amount (lb/day)	Weekly Average Conc. (mg/l)	Weekly Average Amount (lb/day)	Daily Maximum Conc. (mg/l)	Daily Minimum Percent Removal	Measurement Frequency	Sample Type	Sampling Point
CBOD$_5$	5	83	7.5	125	10	40	3/week	composite	effluent
(May 1 - Oct. 31)	Report	—	—	—	Report	—	3/week	composite	influent
CBOD$_5$	15	250	20	333	25	40	3/week	composite	effluent
(Nov. 1 - April 30)	Report	—	—	—	Report	—	3/week	composite	influent
Ammonia as N	1.2	20	1.8	30	2.4	—	3/week	composite	effluent
(May 1 - Oct. 31)									
Ammonia as N	2.8	47	4.2	70	5.6	—	3/week	composite	effluent
(Nov. 1 - April 30)									
Total Nitrogen* (May 1 - Oct. 31)	Report	—	—	—	Report	—	2/month	composite	effluent
Total Phosphorous* (May 1 - Oct. 31)	Report	—	—	—	Report	—	2/month	composite	effluent
Suspended Solids	30	500	40	667	45	40	3/week	composite	effluent
(May 1 - Oct. 31)	Report	—	—	—	Report	—	3/week	composite	influent
Suspended Solids							3/week	composite	effluent
(Nov. 1 - April 30)	Report	—	—	—	Report	—	3/week	composite	influent
Sanitary Sewer Overflows, Total Occurrences	Report						continuous	visual	NA
Dry Weather Overflows, Total Occurrences	Report						continuous	visual	NA
Bypass of Treatment, Total Occurrences	Report						continuous	visual	NA

Note: The permittee shall achieve 85% removal of $CBOD_5$ and TSS on a monthly average basis. The permittee shall report all instances of overflow and/or bypasses. See Part 2.3.3.a for the definition of overflow and Part 1.3.5.1 for reporting requirements.

Note: Unless elsewhere specified, summer months are May through October; winter months are November through April.

Note: See Part 1.2.3 for test procedures.

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Effluent Characteristics	Effluent Limitations			Monitoring Requirements		
	Monthly Average	Daily Minimum	Daily Maximum	Measurement Frequency	Sample Type	Sampling Point
*E. coli**	126/100 ml (see the following paragraphs)	—	487 or 941/100 ml	3/week	grab	effluent
Chlorine residual (Total)	—	—	0.02 mg/l instantaneous	5/week	grab	effluent
Settleable solids	—	—	1.0 ml/l	5/week	grab	effluent
Dissolved oxygen	—	6.0 mg/l instantaneous	—	5/week	grab	effluent
pH (Standard Units)	—	6.5.0	9.0	5/week	grab	effluent
Flow (MGD)	Report	—	Report	7/week	continuous	influent
	Report	—	Report	7/week	continuous	effluent
Mercury, Total	mg/l	— —	— —	1/month	grab	effluent
Cyanide, Total	mg/l	— —	— —	1/month	grab	effluent
48 hr LC_{50}	Survival in % effluent			1/quarter	grab	effluent
IC_{25}	Survival, reproduction and growth in 97% effluent			1/quarter	composite	effluent

Note: See Part 3.4 for biomonitoring test and reporting requirements. See next page for percent removal calculations.

Note: See Part 1.2.3 for test procedures.

* In the absence of a method in 40 CFR, Part 136 for measuring *E. coli* in effluent matrices, the permittee shall use methods proposed or added to Part 136 for measuring *E. coli* in ambient water.

Total residual chlorine (TRC) monitoring shall be applicable when chlorine, bromine, or any other oxidants are added. The acceptable methods for analysis of TRC are any methods specified in Title 40 CFR, Part 136 as amended. The method detection level (MDL) for TRC shall not exceed 0.05 mg/l unless the permittee demonstrates that its MDL is higher. The permittee shall retain the documentation that justifies the higher MDL and have it available for review upon request. In cases where the permit limit is less that the MDL, the reporting of TRC at less than the MDL shall be interpreted to constitute compliance with the permit.

The wastewater discharge must be disinfected to the extent that viable coliform organisms are effectively eliminated. The concentration of the E. *coli* group after disinfection shall not exceed 126 cfu per 100 ml as the geometric mean calculated on the actual number of samples collected and tested for E. *coli* within the required reporting period. The permittee may collect more samples than specified as the monitoring frequency. Samples may not be collected at intervals of less than 12 hours. For the purpose of determining the geometric mean, individual samples having an *E. coli* group concentration of less than one (1) per 100 ml shall be considered as having a concentration of one (1) per 100 ml. In addition, the concentration of the *E. coli* group in any individual sample shall not exceed a specified maximum amount. A maximum daily limit of 487 colonies per 100 ml applies to lakes and Tier II waters. A maximum daily limit of 941 colonies per 100 ml applies to all other recreational waters.

There shall be no distinctly visible floating scum, oil or other matter contained in the wastewater discharge. The wastewater discharge must not cause an objectionable color contrast in the receiving stream.

The wastewater discharge shall not contain pollutants in quantities that will be hazardous or otherwise detrimental to humans, livestock, wildlife, plant life, or fish and aquatic life in the receiving stream.

Sludge or any other material removed by any treatment works must be disposed of in a manner that prevents its entrance into or pollution of any surface or subsurface waters. Additionally, the disposal of such sludge or other material must be in compliance with the Tennessee Solid Waste Disposal Act, TCA 68-31-101 et seq. and the Tennessee Hazardous Waste Management Act, TCA 68-46-101 et seq.

For the purpose of evaluating compliance with the permit limits established herein, where certain limits are below the State of Tennessee published required detection levels (RDLs) for any given effluent characteristics, the results of analyses below the RDL shall be reported as Below Detection Level (BDL), unless in specific cases other detection limits are demonstrated to be the best achievable because of the particular nature of the wastewater being analyzed.

For $CBOD_5$ and TSS, the treatment facility shall demonstrate a minimum of 85% removal efficiency on a monthly average basis. This is calculated by determining an average of all daily influent concentrations and comparing this to an average of all daily effluent concentrations. The formula for this calculation is as follows:

$$\left[1 - \frac{\text{average of daily effluent concentration}}{\text{average of daily influent concentration}} \right] \times 100\% \quad = \%\ \text{removal}$$

The treatment facility will also demonstrate 40% minimum removal of the $CBOD_5$ and TSS based upon each daily composite sample. The formula for this calculation is as follows:

$$\left[1 - \frac{\text{daily effluent concentration}}{\text{daily influent concentration}}\right] \times 100\% \quad = \%\ \text{removal}$$

1.2. MONITORING PROCEDURES

1.2.1. Representative Sampling

Appropriate flow measurement devices and methods consistent with accepted scientific practices shall be selected and used to insure the accuracy and reliability of measurements of the volume of monitored discharges. The devices shall be installed, calibrated and maintained to insure that the accuracy of the measurements is consistent with accepted capability of that type of device. Devices selected shall be capable of measuring flows with a maximum deviation of less than plus or minus 10% from the true discharge rates throughout the range of expected discharge volumes.

Samples and measurements taken in compliance with the monitoring requirements specified above shall be representative of the volume and nature of the monitored discharge, and shall be taken at the following location(s):

Influent samples must be collected prior to mixing with any other wastewater being returned to the head of the plant, such as sludge return. Those systems with more than one influent line must collect samples from each and proportion the results by the flow from each line.

Effluent samples must be representative of the wastewater being discharged and collected prior to mixing with any other discharge or the receiving stream. This can be a different point for different parameters, but must be after all treatment for that parameter or all expected change:

a. $CBOD_5$ samples can be collected before disinfection to avoid having to seed the samples and dechlorinate if chlorine is used.

b. The chlorine residual must be measured after the chlorine contact chamber and any dechlorination. It may be to the advantage of the permittee to measure at the end of any long outfall lines.

c. Samples for *E. Coli* can be collected at any point between disinfection and the actual discharge.

d. The dissolved oxygen can drop in the outfall line; therefore, D.O. measurements are required at the discharge end of outfall lines greater than one mile long. Systems with outfall lines less than one mile may measure dissolved oxygen as the wastewater leaves the treatment facility. For systems with dechlorination, dissolved oxygen must be measured after this step and as close to the end of the outfall line as possible.

e. Total suspended solids and settleable solids can be collected at any point after the final clarifier.

f. Biomonitoring tests (if required) shall be conducted on final effluent.

1.2.2. Sampling Frequency

Where the permit requires sampling and monitoring of a particular effluent characteristic(s) at a frequency of less than once per day or daily, the permittee is precluded from marking the “No Discharge” block on the Discharge Monitoring Report if there has been any discharge from that particular outfall during the period which coincides with the required monitoring frequency; i.e. if the required monitoring frequency is once per month or 1/month, the monitoring period is one month, and if the discharge occurs during only one day in that period then the permittee must sample on that day and report the results of analyses accordingly.

1.2.3. Test Procedures

a. Test procedures for the analysis of pollutants shall conform to regulations published pursuant to Section 304 (h) of the Clean Water Act (the "Act"), as amended, under which such procedures may be required.

b. Unless otherwise noted in the permit, all pollutant parameters shall be determined according to methods prescribed in Title 40, CFR, Part 136, as amended, promulgated pursuant to Section 304 (h) of the Act.

c. In the absence of a method in 40 CFR, Part 136 for measuring *E. coli* in effluent matrices, the permittee shall use methods proposed or added to Part 136 for measuring *E. coli* in ambient water. The Division does recognize the use of EPA Method 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium) for monitoring and reporting as required in the permit limits table(s).

d. Composite samples must be proportioned by flow at time of sampling. Aliquots may be collected manually or automatically. The sample aliquots must be maintained at 4 degrees Celsius during the compositing period.

1.2.4. Recording of Results

For each measurement or sample taken pursuant to the requirements of this permit, the permittee shall record the following information:

a. The exact place, date and time of sampling;

b. The exact person(s) collecting samples;

c. The dates and times the analyses were performed;

d. The person(s) or laboratory who performed the analyses;

e. The analytical techniques or methods used, and;

f. The results of all required analyses.

1.2.5. Records Retention

All records and information resulting from the monitoring activities required by this permit including all records of analyses performed and calibration and maintenance of instrumentation shall be retained for a minimum of three (3) years, or longer, if requested by the Division of Water Pollution Control.

1.3. REPORTING

1.3.1. Monitoring Results

Monitoring results shall be recorded monthly and submitted monthly using Discharge Monitoring Report (DMR) forms or an electronic program supplied by the Division of Water Pollution Control. Submittals shall be postmarked or sent electronically no later than 15 days after the completion of the reporting period. The top two copies of each report are to be submitted. A copy should be retained for the permittee's files. DMRs and any communication regarding compliance with the conditions of this permit must be sent to:

TENNESSEE DEPT. OF ENVIRONMENT & CONSERVATION
DIVISION OF WATER POLLUTION CONTROL
COMPLIANCE REVIEW SECTION
401 CHURCH STREET
L & C ANNEX 6TH FLOOR
NASHVILLE TN 37243-1534

The first DMR is due on the 15th of the month following permit effectiveness.

DMRs and any other report or information submitted to the division must be signed and certified by a responsible corporate officer as defined in 40 CFR 122.22, a general partner or proprietor, or a principal municipal executive officer or ranking elected official, or his duly authorized representative. Such authorization must be submitted in writing and must explain the duties and responsibilities of the authorized representative.

The electronic submission of DMRs will be accepted only if approved in writing by the division. For purposes of determining compliance with this permit, data submitted in electronic format is legally equivalent to data submitted on signed and certified DMR forms.

1.3.2. Additional Monitoring by Permittee

If the permittee monitors any pollutant specifically limited by this permit more frequently than required at the location(s) designated, using approved analytical methods as specified herein, the results of such monitoring shall be included in the calculation and reporting of the values required in the DMR form. Such increased frequency shall also be indicated on the form.

1.3.3. Falsifying Results and/or Reports

Knowingly making any false statement on any report required by this permit or falsifying any result may result in the imposition of criminal penalties as provided for in Section 309 of the Federal Water Pollution Control Act, as amended, and in Section 69-3-115 of the Tennessee Water Quality Control Act.

1.3.4. Monthly Report of Operation

Monthly operational reports shall be submitted on standard forms to the appropriate Division of Water Pollution Control Environmental Field Office in Jackson, Nashville, Chattanooga, Columbia, Cookeville, Memphis, Johnson City, or Knoxville. Reports shall be submitted by the 15th day of the month following data collection.

1.3.5. Bypass and Overflow Reporting

1.3.5.1. Report Requirements

A summary report of known or suspected instances of overflows in the collection system or bypass of wastewater treatment facilities shall accompany the Discharge Monitoring Report. The report must contain the date and duration of the instances of overflow and/or bypassing and the estimated quantity of wastewater released and/or bypassed.

The report must also detail activities undertaken during the reporting period to (1) determine if overflow is occurring in the collection system, (2) correct those known or suspected overflow points and (3) prevent future or possible overflows and any resulting bypassing at the treatment facility.

On the DMR, the permittee must report the number of sanitary sewer overflows, dry-weather overflows and in-plant bypasses separately. Three lines must be used on the DMR form, one for sanitary sewer overflows, one for dry-weather overflows and one for in-plant bypasses.

1.3.5.2. Anticipated Bypass Notification

If, because of unavoidable maintenance or construction, the permittee has need to create an in-plant bypass which would cause an effluent violation, the permittee must notify the division as soon as possible, but in any case, no later than 10 days prior to the date of the bypass.

1.3.6. Reporting Less Than Detection

A permit limit may be less than the accepted detection level. If the samples are below the detection level, then report “BDL” or “NODI =B” on the DMRs. The permittee must use the correct detection levels in all analytical testing required in the permit. The required detection levels are listed in the Rules of the Department of

Environment and Conservation, Division of Water Pollution Control, Chapter 1200-4-3-.05(8).

For example, if the limit is 0.02 mg/l with a detection level of 0.05 mg/l and detection is shown; 0.05 mg/l must be reported. In contrast, if nothing is detected reporting “BDL” or “NODI =B” is acceptable.

1.4. COMPLIANCE WITH SECTION 208

The limits and conditions in this permit shall require compliance with an area-wide waste treatment plan (208 Water Quality Management Plan) where such approved plan is applicable.

1.5. REOPENER CLAUSE

This permit shall be modified, or alternatively revoked and reissued, to comply with any applicable effluent standard or limitation issued or approved under Sections 301(b)(2)(C) and (D), 307(a)(2) and 405(d)(2)(D) of the Clean Water Act, as amended, if the effluent standard, limitation or sludge disposal requirement so issued or approved:

a. Contains different conditions or is otherwise more stringent than any condition in the permit; or

b. Controls any pollutant or disposal method not addressed in the permit.

The permit as modified or reissued under this paragraph shall also contain any other requirements of the Act then applicable.

2.0. GENERAL PERMIT REQUIREMENTS

2.1. GENERAL PROVISIONS

2.1.1. Duty to Reapply

Permittee is not authorized to discharge after the expiration date of this permit. In order to receive authorization to discharge beyond the expiration date, the permittee shall submit such information and forms as are required to the Director of Water Pollution Control (the "director") no later than 180 days prior to the expiration date. Such forms shall be properly signed and certified.

2.1.2. Right of Entry

The permittee shall allow the director, the Regional Administrator of the U.S. Environmental Protection Agency, or their authorized representatives, upon the presentation of credentials:

a. To enter upon the permittee's premises where an effluent source is located or where records are required to be kept under the terms and conditions of this permit, and at reasonable times to copy these records;

b. To inspect at reasonable times any monitoring equipment or method or any collection, treatment, pollution management, or discharge facilities required under this permit; and

c. To sample at reasonable times any discharge of pollutants.

2.1.3. Availability of Reports

Except for data determined to be confidential under Section 308 of the Federal Water Pollution Control Act, as amended, all reports prepared in accordance with the terms of this permit shall be available for public inspection at the offices of the Division of Water Pollution Control. As required by the Federal Act, effluent data shall not be considered confidential.

2.1.4. Proper Operation and Maintenance

a. The permittee shall at all times properly operate and maintain all facilities and systems (and related appurtenances) for collection and treatment which are installed or used by the permittee to achieve compliance with the terms and conditions of this permit. Proper operation and maintenance also includes adequate laboratory and process controls and appropriate quality assurance procedures. This provision requires the operation of backup or auxiliary facilities or similar systems, which are installed by a permittee only when the operation is necessary to achieve compliance with the conditions of the permit. Backup continuous pH and flow monitoring equipment are not required.

b. Dilution water shall not be added to comply with effluent requirements to achieve BCT, BPT, BAT and or other technology based effluent limitations such as those in State of Tennessee Rule 1200-4-5-.03.

2.1.5. Treatment Facility Failure (Industrial Sources)

The permittee, in order to maintain compliance with this permit, shall control production, all discharges, or both, upon reduction, loss, or failure of the treatment facility, until the facility is restored or an alternative method of treatment is provided. This requirement applies in such situations as the reduction, loss, or failure of the primary source of power.

2.1.6. Property Rights

The issuance of this permit does not convey any property rights in either real or personal property, or any exclusive privileges, nor does it authorize any injury to private property or any invasion of personal rights, nor any infringement of federal, state, or local laws or regulations.

2.1.7. Severability

The provisions of this permit are severable. If any provision of this permit due to any circumstance, is held invalid, then the application of such provision to other circumstances and to the remainder of this permit shall not be affected thereby.

2.1.8. Other Information

If the permittee becomes aware of failure to submit any relevant facts in a permit application, or of submission of incorrect information in a permit application or in any report to the director, then the permittee shall promptly submit such facts or information.

2.2. CHANGES AFFECTING THE PERMIT

2.2.1. Planned Changes

The permittee shall give notice to the director as soon as possible of any planned physical alterations or additions to the permitted facility. Notice is required only when:

a. The alteration or addition to a permitted facility may meet one of the criteria for determining whether a facility is a new source in 40 CFR 122.29(b); or

b. The alteration or addition could significantly change the nature or increase the quantity of pollutants discharged. This notification applies to pollutants, which are subject neither to effluent limitations in the permit, nor to notification requirements under 40 CFR 122.42(a)(1).

2.2.2. Permit Modification, Revocation, or Termination

a. This permit may be modified, revoked and reissued, or terminated for cause as described in 40 CFR 122.62 and 122.64, Federal Register, Volume 49, No. 188 (Wednesday, September 26, 1984), as amended.

b. The permittee shall furnish to the director, within a reasonable time, any information which the director may request to determine whether cause exists for modifying, revoking and reissuing, or terminating this permit, or to determine compliance with this permit. The permittee shall also furnish to the director, upon request, copies of records required to be kept by this permit.

c. If any applicable effluent standard or prohibition (including any schedule of compliance specified in such effluent standard or prohibition) is established for any toxic pollutant under Section 307(a) of the Federal Water Pollution Control Act, as amended, the director shall modify or revoke and reissue the permit to conform to the prohibition or to the effluent standard, providing that the effluent standard is more stringent than the limitation in the permit on the toxic pollutant. The permittee shall comply with these effluent standards or prohibitions within the time provided in the regulations that establish these standards or prohibitions, even if the permit has not yet been modified or revoked and reissued to incorporate the requirement.

d. The filing of a request by the permittee for a modification, revocation, reissuance, termination, or notification of planned changes or anticipated noncompliance does not halt any permit condition.

2.2.3. Change of Ownership

This permit may be transferred to another party (provided there are neither modifications to the facility or its operations, nor any other changes which might affect the permit limits and conditions contained in the permit) by the permittee if:

a. The permittee notifies the director of the proposed transfer at least 30 days in advance of the proposed transfer date;

b. The notice includes a written agreement between the existing and new permittees containing a specified date for transfer of permit responsibility, coverage, and liability between them; and

c. The director, within 30 days, does not notify the current permittee and the new permittee of his intent to modify, revoke or reissue, or terminate the permit and to require that a new application be filed rather than agreeing to the transfer of the permit.

Pursuant to the requirements of 40 CFR 122.61, concerning transfer of ownership, the permittee must provide the following information to the division in their formal notice of intent to transfer ownership: 1) the NPDES permit number of the subject permit; 2) the effective date of the proposed transfer; 3) the name and address of the transferor; 4) the name and address of the transferee; 5) the names of the responsible parties for both the transferor and transferee; 6) a statement that the transferee assumes responsibility for the subject NPDES permit; 7) a statement that the transferor relinquishes responsibility for the subject NPDES permit; 8) the signatures of the responsible parties for both the transferor and transferee pursuant to the requirements of 40 CFR 122.22(a), “Signatories to permit applications”; and, 9) a statement regarding any proposed modifications to the facility, its operations, or any other changes which might affect the permit limits and conditions contained in the permit.

2.2.4. Change of Mailing Address

The permittee shall promptly provide to the director written notice of any change of mailing address. In the absence of such notice the original address of the permittee will be assumed to be correct.

2.3. NONCOMPLIANCE

2.3.1. Effect of Noncompliance

All discharges shall be consistent with the terms and conditions of this permit. Any permit noncompliance constitutes a violation of applicable state and federal laws and is grounds for enforcement action, permit termination, permit modification, or denial of permit reissuance.

2.3.2. Reporting of Noncompliance

a. 24-Hour Reporting

In the case of any noncompliance which could cause a threat to public drinking supplies, or any other discharge which could constitute a threat to human health or the environment, the required notice of non-compliance shall be provided to the Division of Water Pollution Control in the appropriate Environmental Field Office within 24-hours from the time the permittee becomes aware of the circumstances. (The Environmental Field Office should be contacted for names and phone numbers of environmental response team).

A written submission must be provided within five days of the time the permittee becomes aware of the circumstances unless the director on a case-by-case basis waives this requirement. The permittee shall provide the director with the following information:

i. A description of the discharge and cause of noncompliance;

ii. The period of noncompliance, including exact dates and times or, if not corrected, the anticipated time the noncompliance is expected to continue; and

iii. The steps being taken to reduce, eliminate, and prevent recurrence of the noncomplying discharge.

b. Scheduled Reporting

For instances of noncompliance which are not reported under subparagraph 2.3.2.a above, the permittee shall report the noncompliance on the Discharge Monitoring Report. The report shall contain all information concerning the steps taken, or planned, to reduce, eliminate, and prevent recurrence of the violation and the anticipated time the violation is expected to continue.

2.3.3. Overflow

a. "**Overflow**" means any release of sewage from any portion of the collection, transmission, or treatment system other than through permitted outfalls.

b. Overflows are prohibited.

c. The permittee shall operate the collection system so as to avoid overflows. No new or additional flows shall be added upstream of any point in the collection system, which experiences chronic overflows (greater than 5 events per year) or would otherwise overload any portion of the system.

d. Unless there is specific enforcement action to the contrary, the permittee is relieved of this requirement after: 1) an authorized representative of the Commissioner of the Department of Environment and Conservation has approved an engineering report and construction plans and specifications prepared in accordance with accepted engineering practices for correction of the problem; 2) the correction work is underway; and 3) the cumulative, peak-design, flows potentially added from new connections and line extensions upstream of any chronic overflow point are less than or proportional to the amount of inflow and infiltration removal documented upstream of that point. The inflow and infiltration reduction must be measured by the permittee using practices that are customary in the environmental engineering field and reported in an attachment to a Monthly Operating Report submitted to the local TDEC Environmental Field Office. The data measurement period shall be sufficient to account for seasonal rainfall patterns and seasonal groundwater table elevations.

e. In the event that more than 5 overflows have occurred from a single point in the collection system for reasons that may not warrant the self-imposed moratorium or completion of the actions identified in this paragraph, the permittee may request a meeting with the Division of Water Pollution Control EFO staff to petition for a waiver based on mitigating evidence.

2.3.4. Upset

a. "***Upset***" means an exceptional incident in which there is unintentional and temporary noncompliance with technology-based effluent limitations because of factors beyond the reasonable control of the permittee. An upset does not include noncompliance to the extent caused by operational error, improperly designed treatment facilities, inadequate treatment facilities, lack of preventive maintenance, or careless or improper operation.

b. An upset shall constitute an affirmative defense to an action brought for noncompliance with such technology-based permit effluent limitations if the permittee demonstrates, through properly signed, contemporaneous operating logs, or other relevant evidence that:

   i. An upset occurred and that the permittee can identify the cause(s) of the upset;

   ii. The permitted facility was at the time being operated in a prudent and workman-like manner and in compliance with proper operation and maintenance procedures;

   iii. The permittee submitted information required under "Reporting of Noncompliance" within 24-hours of becoming aware of the upset (if this information is provided orally, a written submission must be provided within five days); and

   iv. The permittee complied with any remedial measures required under "Adverse Impact."

2.3.5. Adverse Impact

The permittee shall take all reasonable steps to minimize any adverse impact to the waters of Tennessee resulting from noncompliance with this permit, including such accelerated or additional monitoring as necessary to determine the nature and impact of the noncomplying discharge. It shall not be a defense for the permittee in an enforcement action that it would have been necessary to halt or reduce the permitted activity in order to maintain compliance with the conditions of this permit.

2.3.6. Bypass

a. "***Bypass***" is the intentional diversion of wastewater away from any portion of a treatment facility. "Severe property damage" means substantial physical damage to property, damage to the treatment facilities which would cause them to become inoperable, or substantial and permanent loss of natural resources which can reasonably be expected to occur in the absence of a bypass. Severe property damage does not mean economic loss caused by delays in production.

b. Bypasses are prohibited unless all of the following 3 conditions are met:

   i. The bypass is unavoidable to prevent loss of life, personal injury, or severe property damage;

   ii. There are no feasible alternatives to bypass, such as the construction and use of auxiliary treatment facilities, retention of untreated wastes, or maintenance during normal periods of equipment downtime. This condition is not satisfied if adequate back-up equipment should have been installed in the exercise of reasonable engineering judgment to prevent a bypass, which occurred during normal periods of equipment downtime or preventative maintenance;

   iii. The permittee submits notice of an unanticipated bypass to the Division of Water Pollution Control in the appropriate Environmental Field Office within 24 hours of becoming aware of the bypass (if this information is provided orally, a written submission must be provided within five days). When the need for the bypass is foreseeable, prior notification shall be submitted to the director, if possible, at least 10 days before the date of the bypass.

c. Bypasses not exceeding permit limitations are allowed **only** if the bypass is necessary for essential maintenance to assure efficient operation. All other bypasses are prohibited. Allowable bypasses not exceeding limitations are not subject to the reporting requirements of 2.3.6.b.iii, above.

2.3.7. Washout

a. For domestic wastewater plants only, a "washout" shall be defined as loss of Mixed Liquor Suspended Solids (MLSS) of 30.00% or more. This refers to the MLSS in the aeration basin(s) only. This does not include MLSS decrease due to solids wasting to the sludge disposal system. A washout can be caused by improper operation or from peak flows due to infiltration and inflow.

b. A washout is prohibited. If a washout occurs the permittee must report the incident to the Division of Water Pollution Control in the appropriate Environmental Field Office within 24 hours by telephone. A written submission must be provided within five days. The washout must be noted on the discharge monitoring report. Each day of a washout is a separate violation.

2.4. LIABILITIES

2.4.1. Civil and Criminal Liability

Except as provided in permit conditions for "***Bypassing*,**" "***Overflow,***" and "***Upset*,**" nothing in this permit shall be construed to relieve the permittee from civil or criminal penalties for noncompliance. Notwithstanding this permit, the permittee shall remain liable for any damages sustained by the State of Tennessee, including but not limited to fish kills and losses of aquatic life and/or wildlife, as a result of the discharge of wastewater to any surface or subsurface waters. Additionally, notwithstanding this Permit, it shall be the responsibility of the permittee to conduct its wastewater treatment and/or discharge activities in a manner such that public or private nuisances or health hazards will not be created.

2.4.2. Liability Under State Law

Nothing in this permit shall be construed to preclude the institution of any legal action or relieve the permittee from any responsibilities, liabilities, or penalties established pursuant to any applicable state law or the Federal Water Pollution Control Act, as amended.

3.0. PERMIT SPECIFIC REQUIREMENTS

3.1. CERTIFIED OPERATOR

The waste treatment facilities shall be operated under the supervision of a Grade 3 certified wastewater treatment operator and the collection system operated under the supervision of a Grade 1 Collection System certified operator in accordance with the Water Environmental Health Act of 1984.

Paragraph 1a-c applies if the STP does NOT have an approved pretreatment program:
Paragraph 2a-c applies if the pretreatment program is inactive:
Paragraph 3a-d applies if the STP has an approved or developing pretreatment program:

3.2. POTW PRETREATMENT PROGRAM GENERAL PROVISIONS

As an update of information previously submitted to the division, the permittee will undertake the following activity.

(If developing, replace the above sentence with the one below, and delete the 120-day IWS submission requirement in 1c.h.)

Requirements of Section III.B. shall apply after the division director or pretreatment coordinator has approved the pretreatment program by letter.

1a. The permittee shall submit the results of an Industrial Waste Survey (IWS) in accordance with 40 CFR 403.8(f)(2)(i), including any industrial users (IU) covered under Section 301(i)(2) of the Act. As much information as possible must be obtained relative to the character and volume of pollutants contributed to the POTW by the IUs. This information will be submitted to the Division of Water Pollution Control, Pretreatment Section within one hundred twenty (120) days of the effective date of this permit. Development of a pretreatment program may be required after completion of the industrial user review. All requirements and conditions of the pretreatment program are enforceable through the NPDES permit.

2a. The current pretreatment program is in the inactive stage. The program will remain inactive as long as no significant industries discharge into the collection system. Should a significant industrial user request permission to discharge into the --- system, then the City must request that the division reactivate the pretreatment program. This must be done prior to the industrial discharge taking place.

The permittee shall submit the results of an Industrial Waste Survey (IWS) in accordance with 40 CFR 403.8(f)(2)(i), including any industrial users (IU) covered under Section 301(i)(2) of the Act. As much information as possible must be obtained relative to the character and volume of pollutants contributed to the

POTW by the IUs. This information will be submitted to the Division of Water Pollution Control, Pretreatment Section within one hundred twenty (120) days of the effective date of this permit. Development of a pretreatment program may be required after completion of the industrial user review. All requirements and conditions of the pretreatment program are enforceable through the NPDES permit.

3a. The permittee has been delegated the primary responsibility and therefore becomes the "control authority" for enforcing the 40 CFR 403 General Pretreatment Regulations. Where multiple plants are concerned the permittee is responsible for the Pretreatment Program for all plants within its jurisdiction. The permittee shall implement and enforce the Industrial Pretreatment Program in accordance with Section 403(b)(8) of the Clean Water Act, the Federal Pretreatment Regulations 40 CFR 403, Tennessee Water Quality Control Act Part 63-3-123 through 63-3-128, and the legal authorities, policies, procedures, and financial provisions contained in its approved Pretreatment Program, except to the extent this permit imposed stricter requirements. Such implementation shall require but not limit the permittee to do the following:

i. Carry out inspection, surveillance, and monitoring procedures which will determine, independent of information supplied by the industrial user (IU), whether the IU is in compliance with the pretreatment standards;

ii. Require development, as necessary, of compliance schedules for each IU for the installation of control technologies to meet applicable pretreatment standards;

iii. Require all industrial users to comply with all applicable monitoring and reporting requirements outlined in the approved pretreatment program and IU permit;

iv. Maintain and update, as necessary, records identifying the nature and character of industrial user discharges, and retain such records for a minimum of three (3) years;

v. Obtain appropriate remedies for noncompliance by an IU with any pretreatment standard and/or requirement;

vi. Publish annually, pursuant to 40 CFR 403.8 (f)(2)(vii), a list of industrial users that have significantly violated pretreatment requirements and standards during the previous twelve-month period.

vii. Maintain an adequate revenue structure for continued operation of the pretreatment program.

viii. Update its Industrial Waste Survey at least once every five years. Results of this update shall be submitted to the Division of Water Pollution Control, Pretreatment Section within 120 days of the effective date of this permit.

ix. Submit a written technical evaluation of the need to revise local limits within 120 days of the effective date of this permit to the state pretreatment program coordinator. The evaluation shall include the most recent pass-through limits proposed by the division. The technical evaluation shall be based on practical and specialized knowledge of the local program and not be limited by a specified written format.

b. The permittee shall enforce 40 CFR 403.5, "prohibited discharges". Pollutants introduced into the POTW by a non-domestic source shall not cause pass through or interference as defined in 40 CFR Part 403.3. These general prohibitions and the specific prohibitions in this section apply to all non-domestic sources introducing pollutants into the POTW whether the source is subject to other National Pretreatment Standards or any state or local pretreatment requirements.

Specific prohibitions. Under no circumstances shall the permittee allow introduction of the following wastes in the waste treatment system:

i. Pollutants which create a fire or explosion hazard in the POTW;

ii. Pollutants which will cause corrosive structural damage to the treatment works, but in no case discharges with pH less than 5.0 unless the system is specifically designed to accept such discharges.

iii. Solid or viscous pollutants in amounts which will cause obstruction to the flow in the treatment system resulting in interference.

iv. Any pollutant, including oxygen-demanding pollutants (BOD, etc.) released in a discharge at a flow rate and/or pollutant concentration which will cause interference with the treatment works.

v. Heat in amounts which will inhibit biological activity in the treatment works resulting in interference, but in no case heat in such quantities that the temperature at the treatment works exceeds 40°C (104°F) unless the works are designed to accommodate such heat.

vi. Any priority pollutant in amounts that will contaminate the treatment works sludge.

vii. Petroleum oil, nonbiodegradable cutting oil, or products of mineral oil origin in amounts that will cause interference or pass through;

viii. 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;

ix. Any trucked or hauled pollutants except at discharge points designated by the POTW.

c. The permittee shall notify the Tennessee Division of Water Pollution Control of any of the following changes in user discharge to the system no later than 30 days prior to change of discharge:

   i. New introductions into such works of pollutants from any source which would be a new source as defined in Section 306 of the Act if such source were discharging pollutants.

   ii. New introductions of pollutants into such works from a source which would be subject to Section 301 of the "Federal Water Quality Act as Amended" if it were discharging such pollutants.

   iii. A substantial change in volume or character of pollutants being introduced into such works by a source already discharging pollutants into such works at the time the permit is issued.

   This notice will include information on the quantity and quality of the wastewater introduced by the new source into the publicly owned treatment works, and on any anticipated impact on the effluent discharged from such works. If this discharge necessitates a revision of the current NPDES permit or pass-through guidelines, discharge by this source is prohibited until the Tennessee Division of Water Pollution Control gives final authorization.

d. Reporting Requirements

   The permittee shall provide a semiannual report briefly describing the permittee's pretreatment program activities over the previous six-month period. Reporting periods shall end on the last day of the months of March and September. The report shall be submitted to the Division of Water Pollution Control, Central Office and a copy to the appropriate Environmental Field Office no later than the 28th day of the month following each reporting period. For control authorities with multiple STPs, one report should be submitted with a separate Form 1 for each STP. Each report shall conform to the format set forth in the State POTW Pretreatment Semiannual Report Package which contains information regarding:

   i. An updated listing of the permittee's industrial users.

   ii. Results of sampling of the influent and effluent of the wastewater treatment plant. At least once each reporting period, the permittee shall analyze the wastewater treatment plant influent and effluent for the following pollutants, using the prescribed sampling procedures:

(approved and developing programs)

Pollutant	**Sample Type**
chromium	24-hour composite
copper	24-hour composite
lead	24-hour composite
nickel	24-hour composite
zinc	24-hour composite
cadmium	24-hour composite
mercury	24-hour composite
silver	24-hour composite
total phenols	grab
cyanide	grab

If any particular pollutant is analyzed more frequently than is required, the permittee shall report the maximum and average values on the semiannual report. All upsets, interferences, and pass-through violations must also be reported on the semiannual report, the actions that were taken to determine the causes of the incidents and the steps that have been taken to prevent the incidents from recurring.

At least once during the term of this permit, the permittee shall analyze the effluent from the STP (and report the results in the next regularly scheduled report) for the following pollutants:

chromium, total	silver	phthalates, sum of the following: bis (2-ethylhexyl) phthalate butyl benzylphthalate di-n-butylphthalate diethyl phthalate
copper	benzene	
lead	carbon tetrachloride	
nickel	chloroform	
zinc	ethylbenzene	
cadmium	methylene chloride	tetrachloroethylene
mercury	naphthalene	toluene
phenols, total	1,1,1 trichloroethane	trichloroethylene
cyanide	1,2 trans-dichloroethylene	

iii. Compliance with categorical and local standards, and review of industrial compliance, which includes a summary of the compliance status for all permitted industries. Also included is information on the number and type of major violations of pretreatment regulations, and the actions taken by the POTW to obtain compliance. The effluent from all significant industrial users must be analyzed for the appropriate pollutants at least once per reporting period.

iv. A list of industries in significant non-compliance as published in local newspapers in accordance with the requirements set forth in 40 CFR 403.8(f)(2)(vii).

v. A description of all substantive changes made to the permittee's pretreatment program. Any such changes shall receive prior approval. Substantive changes include, but are not limited to, any change in any ordinance, major modification in the program's administrative structure, local limits, or a change in the method of funding the program.

vi. Summary of permittee's industrial user inspections, which includes information on the number and type of industry inspected. All significant industrial users must be inspected at least once per year.

3.3. SLUDGE MANAGEMENT PRACTICES

a. The permittee must comply with 40 CFR 503 et seq. Sludge shall be sampled and analyzed at a frequency dependant both on the amount of sludge generated annually and on the disposal practice utilized. Whenever sampling and analysis are required by 40 CFR 503, the permittee shall report to the division the quantitative data for the following parameters:

1)	Arsenic	7)	Nickel
2)	Cadmium	8)	Selenium
3)	Copper	9)	Zinc
4)	Lead	10)	Nitrite plus Nitrate, NO_2, + NO_3 as N
5)	Mercury	11)	Total Kjeldahl Nitrogen, as N
6)	Molybdenum	12)	Ammonia, NH_3, as N

This sludge analysis must be submitted by February 19th of each calendar year. This information shall be submitted to the Division of Water Pollution Control, Central Office, 401 Church Street, 6th Floor Annex, Nashville TN 37243-1534, Attention: Sludge Coordinator, Municipal Facilities Section.

b. Land application of sludge shall halt immediately if any of the following concentrations are exceeded:

POLLUTANT	CONCENTRATION (mg/kg[1])
Arsenic	75
Cadmium	85
Zinc	7500
Copper	4300
Lead	840

POLLUTANT	CONCENTRATION (mg/kg[1])
Mercury	57
Molybdenum	75
Nickel	420
Selenium	100

1 Dry Weight Basis

Monthly average pollutant concentrations shall not exceed Table 3 of 40 CFR §503.13. If they are exceeded cumulative pollutant loading rates are to be calculated and recorded and shall not exceed Table 2 of 40 CFR §503.13 for the life of the land application site.

c. If land application is the final disposition of the wasted sludge, the permittee shall provide pathogen reduction, sludge stabilization and comply with land and crop usage controls as listed in 40 CFR Part 503, as authorized by the Clean Water Act. Records must be maintained by the permittee that indicate compliance or non-compliance with this rule. If the permittee is required to report to EPA, copies of all reports should be sent to the division, at the address listed in paragraph 1 of this section.

d. Before land applying municipal sludge the permittee must obtain approvals for each site(s) in writing from the division using the latest revision of Guidelines for Land Application or Surface Disposal of Biosolids, unless the sludge being land applied meets the pollutant concentrations of 40 CFR 503.13(b)(3), the Class A pathogen requirements in 40 CFR 503.32(a), and one of the vector attraction reduction requirements in 40 CFR 503.33 (b)(1) through (b)(8).

e. Reopener: If an applicable "acceptable management practice" or numerical limitation for pollutants in sewage sludge promulgated under Section 405(d)(2) of the Clean Water Act, as amended by the Water Quality Act of 1987, is more stringent than the sludge pollutant limit or acceptable management practice in this permit, or controls a pollutant not limited in this permit, this permit shall be promptly modified or revoked and reissued to conform to the requirements promulgated under Section 405(d)(2). The permittee shall comply with the limitations by no later than the compliance deadline specified in the applicable regulations as required by Section 405(d)(2) of the Clean Water Act.

f. Notice of change in sludge disposal practice: The permittee shall give prior notice to the director of any change planned in the permittee's sludge disposal practice. If land application activities are suspended permanently and sludge disposal moves to a municipal solid waste landfill, the permittee shall contact the local Division of Solid Waste Management office address for other permitting and approvals (see table below):

Division of Solid Waste Management			
Office	**Location**	**Zip Code**	**Phone No.**
Chattanooga	540 McCallie Avenue, Suite 550	37402-2013	(423) 634-5745
Jackson	362 Carriage House Drive	38305-2222	(731) 512-1300
Cookeville	1221 South Willow Avenue	38506	(931) 432-4015
Columbia	2484 Park Plus Drive	38401	(931) 380-3371
Johnson City	2305 Silverdale Road	37601	(423) 854-5400
Knoxville	2700 Middlebrook Pike, Suite 220	37921	(865) 594-6035
Memphis	2510 Mt. Moriah Road, Suite E-645	38115-1511	(901) 368-7939
Nashville	711 R.S. Gass Boulevard	37243-1550	(615) 687-7000

If sludge disposal is to a municipal solid waste landfill:

The current method of sludge disposal is to a municipal solid waste landfill (or co - composting facility). This method of disposal is controlled by the rules of the Tennessee Division of Solid Waste Management (DSWM) and Federal Regulations at 40 CFR 258. If the permittee anticipates changing its disposal practices to either land application or surface disposal, the Division of Water Pollution Control shall be notified prior to the change. A copy of the results of pollutant analyses required by the Tennessee Division of Solid Waste Management (DSWM) and / or 40 CFR 258 shall be submitted to the Division of Water Pollution Control.

Sludge language for lagoon systems:

a. The permittee shall give prior notice to the director of any change planned in the permittee's sludge disposal practice. In the event the --- STP removes any sludge from any lagoon the permittee must comply with 40 CFR 503 et seq.

b. Before land applying municipal sludge the permittee must obtain approvals for each site(s) in writing from the division using the latest revision of Guidelines for Land Application or Surface Disposal of Biosolids, unless the sludge being land applied meets the pollutant concentrations of 40 CFR 503.13(b)(3), the Class A pathogen requirements in 40 CFR 503.32(a), and one of the vector attraction reduction requirements in 40 CFR 503.33 (b)(1) through (b)(8).

c. If sludge disposal moves to a municipal solid waste landfill, the permittee shall contact the local Division of Solid Waste Management office address for other permitting and approvals (see table below):

Division of Solid Waste Management			
Office	**Location**	**Zip Code**	**Phone No.**
Chattanooga	540 McCallie Avenue, Suite 550	37402-2013	(423) 634-5745
Jackson	362 Carriage House Drive	38305-2222	(731) 512-1300
Cookeville	1221 South Willow Avenue	38506	(931) 432-4015
Columbia	2484 Park Plus Drive	38401	(931) 380-3371
Johnson City	2305 Silverdale Road	37601	(423) 854-5400
Knoxville	2700 Middlebrook Pike, Suite 220	37921	(865) 594-6035
Memphis	2510 Mt. Moriah Road, Suite E-645	38115-1511	(901) 368-7939
Nashville	711 R.S. Gass Boulevard	37243-1550	(615) 687-7000

3.4. BIOMONITORING REQUIREMENTS, CHRONIC

The permittee shall conduct a 3-Brood *Ceriodaphnia dubia* Survival and Reproduction Test and a 7-Day Fathead Minnow *(Pimephales promelas)* Larval Survival and Growth Test on samples of final effluent from Outfall 001.

The measured endpoint for toxicity will be the inhibition concentration causing 25% reduction in survival, reproduction and growth (IC_{25}) of the test organisms. The IC_{25} shall be determined based on a 25% reduction as compared to the controls, and as derived from linear interpolation. The average reproduction and growth responses will be determined based on the number of *Ceriodaphnia dubia* or *Pimephales promelas* larvae used to initiate the test.

If the permit limit is 100%, use this table:

Test shall be conducted and its results reported based on appropriate replicates of a total of five serial dilutions and a control, using the percent effluent dilutions as presented in the following table:

Serial Dilutions for Whole Effluent Toxicity (WET) Testing					
Permit Limit (PL)	0.50 X PL	0.25 X PL	0.125 X PL	0.0625 X PL	Control
% effluent					
100	50	25	12.5	6.25	0

If the permit limit is at or above 90%, use this table:
(enter the permit limit in the appropriate field, highlight the entire row, press F9)

Test shall be conducted and its results reported based on appropriate replicates of a total of five serial dilutions and a control, using the percent effluent dilutions as presented in the following table:

Serial Dilutions for Whole Effluent Toxicity (WET) Testing					
100% Effluent	Permit Limit (PL)	0.50 X PL	0.25 X PL	0.125 X PL	Control
% effluent					
100	xx	0.0	0.0	0.0	0

If the permit limit is above 25%, but below 90%, use this table:
(enter the permit limit in the appropriate field, highlight the entire row, press F9)

Test shall be conducted and its results reported based on appropriate replicates of a total of five serial dilutions and a control, using the percent effluent dilutions as presented in the following table:

Serial Dilutions for Whole Effluent Toxicity (WET) Testing					
100% Effluent	(100+PL)/2	Permit Limit (PL)	0.50 X PL	0.25 X PL	Control
% effluent					
100	50	xx	0.0	0.0	0

If the permit limit is at or below 25%, use this table:
(enter the permit limit in the appropriate field, highlight the entire row, press F9)

Test shall be conducted and its results reported based on appropriate replicates of a total of five serial dilutions and a control, using the percent effluent dilutions as presented in the following table:

Serial Dilutions for Whole Effluent Toxicity (WET) Testing					
4 X PL	**2 X PL**	**Permit Limit (PL)**	**0.50 X PL**	**0.25 X PL**	**Control**
% effluent					
0	**0**	**xx**	**0.0**	**0.0**	**0**

The dilution/control water used will be moderately hard water as described in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, EPA-821-R-02-013 (or the most current edition). A chronic standard reference toxicant quality assurance test shall be conducted with each species used in the toxicity tests and the results submitted with the discharge monitoring report. Additionally, the analysis of this multi-concentration test shall include review of the concentration-response relationship to ensure that calculated test results are interpreted appropriately.

Toxicity will be demonstrated if the IC_{25} is less than or equal to the permit limit indicated for each outfall in the above table(s). Toxicity demonstrated by the tests specified herein constitutes a violation of this permit.

All tests will be conducted using a minimum of three 24-hour flow-proportionate composite samples of final effluent collected on days 1, 3 and 5. If, in any control more than 20% of the test organisms die in 7 days, the test (control and effluent) is considered invalid and the test shall be repeated within two (2) weeks. Furthermore, if the results do not meet the acceptability criteria in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, EPA-821-R-02-013 (or the most current edition), or if the required concentration-response review fails to yield a valid relationship per guidance contained in Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing, EPA-821-B-00-004 (or the most current edition), that test shall be repeated. Any test initiated but terminated before completion must also be reported along with a complete explanation for the termination.

USE THIS LANGUAGE WHEN DILUTION RATIO IS GREATER THAN 500 TO 1:

The toxicity tests specified herein shall be conducted yearly (1/yr) for Outfall 001 and begin no later than 90 days from the effective date of this permit. Monitoring frequency will be 1/quarter when a non-categorical Significant Industrial User (SIU) or a Categorical Industrial User (CIU) discharges to the treatment works.

The toxicity tests specified herein shall be conducted quarterly (1/Quarter) for Outfall 001 and begin no later than 90 days from the effective date of this permit.

In the event of a test failure, the permittee must start a follow-up test within 2 weeks and submit results from a follow-up test within 30 days from obtaining initial WET testing results. The follow-up test must be conducted using the same serial dilutions as presented in the corresponding table(s) above. **The follow-up test will not negate an initial failed test. In addition, the failure of a follow-up test will constitute a separate permit violation.**

In the event of 2 consecutive test failures or 3 test failures within a 12-month period for the same outfall, the permittee must initiate a Toxicity Identification Evaluation/Toxicity Reduction Evaluation (TIE/TRE) study within 30 days and so notify the division by letter. This notification shall include a schedule of activities for the initial investigation of that outfall. **During the term of the TIE/TRE study, the frequency of biomonitoring shall be once every three months.** Additionally, the permittee shall submit progress reports once every three months throughout the term of the TIE/TRE study. The toxicity must be reduced to allowable limits for that outfall within 2 years of initiation of the TIE/TRE study. Subsequent to the results obtained from the TIE/TRE studies, the permittee may request an extension of the TIE/TRE study period if necessary to conduct further analyses. The final determination of any extension period will be made at the discretion of the division.

The TIE/TRE study may be terminated at any time upon the completion and submission of 2 consecutive tests (for the same outfall) demonstrating compliance. Following the completion of TIE/TRE study, the frequency of monitoring will return to a regular schedule, as defined previously in this section as well in Part I of the permit. **During the course of the TIE/TRE study, the permittee will continue to conduct toxicity testing of the outfall being investigated at the frequency of once every three months but will not be required to perform follow-up tests for that outfall during the period of TIE/TRE study.**

Test procedures, quality assurance practices, determinations of effluent survival/reproduction and survival/growth values, and report formats will be made in accordance with Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, EPA-821-R-02-013, or the most current edition.

Results of all tests, reference toxicant information, copies of raw data sheets, statistical analysis and chemical analyses shall be compiled in a report. The report will be written in accordance with Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, EPA-821-R-02-013, or the most current edition.

Two copies of biomonitoring reports (including follow-up reports) shall be submitted to the division. One copy of the report shall be submitted along with the discharge monitoring report (DMR). The second copy shall be submitted to the local Division of Water Pollution Control office address (see table below):

Division of Water Pollution Control			
Office	**Location**	**Zip Code**	**Phone No.**
Chattanooga	540 McCallie Avenue, Suite 550	37402-2013	(423) 634-5745
Jackson	362 Carriage House Drive	38305-2222	(731) 512-1300
Cookeville	1221 South Willow Avenue	38506	(931) 432-4015
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Johnson City	2305 Silverdale Road	37601	(423) 854-5400
Knoxville	2700 Middlebrook Pike, Suite 220	37921	(865) 594-6035
Memphis	2510 Mt. Moriah Road, Suite E-645	38115-1511	(901) 368-7939
Nashville	711 R.S. Gass Boulevard	37243-1550	(615) 687-7000

3.5. BIOMONITORING REQUIREMENTS, ACUTE

The permittee shall conduct a 48-hour static acute toxicity test on two test species on samples of final effluent from Outfall 001. The test species to be used are Water Fleas (Ceriodaphnia dubia) and Fathead Minnows (Pimephales promelas).

The measured endpoint for toxicity will be the concentration causing 50% lethality (LC50) of the test organisms. The LC50 shall be determined based on a 50% lethality as compared to the controls, and as derived from linear interpolation.

If the permit limit is 100%, use this table:

Test shall be conducted and its results reported based on appropriate replicates of a total of five serial dilutions and a control, using the percent effluent dilutions as presented in the following table:

Serial Dilutions for Whole Effluent Toxicity (WET) Testing					
Permit Limit (PL)	**0.50 X PL**	**0.25 X PL**	**0.125 X PL**	**0.0625 X PL**	**Control**
% effluent					
100	**50**	**25**	**12.5**	**6.25**	**0**

If the permit limit is at or above 90%, use this table:
(enter the permit limit in the appropriate field, highlight the entire row, press F9)

Test shall be conducted and its results reported based on appropriate replicates of a total of five serial dilutions and a control, using the percent effluent dilutions as presented in the following table:

Serial Dilutions for Whole Effluent Toxicity (WET) Testing					
100% Effluent	**Permit Limit (PL)**	**0.50 X PL**	**0.25 X PL**	**0.125 X PL**	**Control**
% effluent					
100	**xx**	**0.0**	**0.0**	**0.0**	**0**

If the permit limit is above 25%, but below 90%, use this table:
(enter the permit limit in the appropriate field, highlight the entire row, press F9)

Test shall be conducted and its results reported based on appropriate replicates of a total of five serial dilutions and a control, using the percent effluent dilutions as presented in the following table:

Serial Dilutions for Whole Effluent Toxicity (WET) Testing					
100% Effluent	**(100+PL)/2**	**Permit Limit (PL)**	**0.50 X PL**	**0.25 X PL**	**Control**
% effluent					
100	**50**	**xx**	**0.0**	**0.0**	**0**

If the permit limit is at or below 25%, use this table:
(enter the permit limit in the appropriate field, highlight the entire row, press F9)

Test shall be conducted and its results reported based on appropriate replicates of a total of five serial dilutions and a control, using the percent effluent dilutions as presented in the following table:

Serial Dilutions for Whole Effluent Toxicity (WET) Testing					
4 X PL	**2 X PL**	**Permit Limit (PL)**	**0.50 X PL**	**0.25 X PL**	**Control**
% effluent					
0	**0**	**xx**	**0.0**	**0.0**	**0**

The dilution/control water used will be moderately hard water as described in Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, EPA-821-R-02-012 (or the most current edition). An acute standard reference toxicant quality assurance test shall be conducted with each species used in the toxicity tests and the results submitted with the discharge monitoring report. Additionally, the analysis of this multi-concentration test shall include review of the concentration-response relationship to ensure that calculated test results are interpreted appropriately.

Toxicity will be demonstrated if the LC50 is less than or equal to the permit limit indicated for each outfall in the above table(s). Toxicity demonstrated by the tests specified herein constitutes a violation of this permit.

All tests will be conducted using four separate grab samples of final effluent, to be used in four separate tests, and shall be collected at evenly spaced (6-hour) intervals over a 24-hour period. If in any control, more than 10% of the test organisms die in 48 hours, the test (control and effluent) is considered invalid and the test shall be repeated within two (2) weeks. Furthermore, if the results do not meet the acceptability criteria in Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, EPA-821-R-02-012 (or the most current edition), if the required concentration-response review fails to yield a valid relationship per guidance contained in Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing, EPA-821-B-00-004 (or the most current edition), that test shall be repeated. Any test initiated but terminated before completion must also be reported along with a complete explanation for the termination.

The toxicity tests specified herein shall be conducted quarterly (1/Quarter) for Outfall 001 and begin no later than 90 days from the effective date of this permit.

In the event of a test failure, the permittee must start a follow-up test within 2 weeks and submit results from a follow-up test within 30 days from obtaining initial WET testing results. The follow-up test must be conducted using the same serial dilutions as presented in the corresponding table(s) above. The follow-up test will not negate an initial failed test. In addition, the failure of a follow-up test will constitute a separate permit violation.

In the event of 2 consecutive test failures or 3 test failures within a 12-month period for the same outfall, the permittee must initiate a Toxicity Identification Evaluation/Toxicity Reduction Evaluation (TIE/TRE) study within 30 days and so notify the division by letter. This notification shall include a schedule of activities for the initial investigation of that outfall. During the term of the TIE/TRE study, the frequency of biomonitoring shall be once every three months. Additionally, the permittee shall submit progress reports once every three months throughout the term of the TIE/TRE study. The toxicity must be reduced to allowable limits for that outfall within 2 years of initiation of the TIE/TRE study. Subsequent to the results obtained from the TIE/TRE studies, the permittee may request an extension of the TIE/TRE study period if necessary to conduct further analyses. The final determination of any extension period will be made at the discretion of the division.

The TIE/TRE study may be terminated at any time upon the completion and submission of 2 consecutive tests (for the same outfall) demonstrating compliance. Following the completion of TIE/TRE study, the frequency of monitoring will return to a regular schedule, as defined previously in this section as well in Part I of the permit. During the course of the TIE/TRE study, the permittee will continue to conduct toxicity testing of the outfall being investigated at the frequency of once every three months but will not be required to perform follow-up tests for that outfall during the period of TIE/TRE study.

Test procedures, quality assurance practices and determination of effluent lethality values will be made in accordance with Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, EPA-821-R-02-012, or the most current edition.

Results of all tests, reference toxicant information, copies of raw data sheets, statistical analysis and chemical analysis shall be compiled in a report. The report shall be written in accordance with Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, EPA-821-R-02-012, or the most current edition.

Two copies of biomonitoring reports (including follow-up reports) shall be submitted to the division. One copy of the report shall be submitted along with the discharge monitoring report (DMR). The second copy shall be submitted to the local Division of Water Pollution Control office address (see table below):

Division of Water Pollution Control			
Office	**Location**	**Zip Code**	**Phone No.**
Chattanooga	540 McCallie Avenue, Suite 550	37402-2013	(423) 634-5745
Jackson	362 Carriage House Drive	38305-2222	(731) 512-1300
Cookeville	1221 South Willow Avenue	38506	(931) 432-4015
Columbia	2484 Park Plus Drive	38401	(931) 380-3371
Johnson City	2305 Silverdale Road	37601	(423) 854-5400
Knoxville	2700 Middlebrook Pike, Suite 220	37921	(865) 594-6035
Memphis	2510 Mt. Moriah Road, Suite E-645	38115-1511	(901) 368-7939
Nashville	711 R.S. Gass Boulevard	37243-1550	(615) 687-7000

3.6. PLACEMENT OF SIGNS

Within sixty (60) days of the effective date of this permit, the permittee shall place and maintain a sign(s) at each outfall and any bypass/overflow point in the collection system. For the purposes of this requirement, any bypass/overflow point that has discharged five (5) or more times in the last year must be so posted. The sign(s) should be clearly visible to the public from the bank and the receiving stream. The <u>minimum</u> sign size should be two feet by two feet (2' x 2') with one-inch (1") letters. The sign should be made of durable material and have a white background with black letters.

The sign(s) are to provide notice to the public as to the nature of the discharge and, in the case of the permitted outfalls, that the discharge is regulated by the Tennessee Department of Environment and Conservation, Division of Water Pollution Control. The following is given as an example of the minimal amount of information that must be included on the sign:

Permitted CSO or unpermitted bypass/overflow point:

UNTREATED WASTEWATER DISCHARGE POINT
--- STP
contact phone number
NPDES Permit NO. TN00-----
TENNESSEE DIVISION OF WATER POLLUTION CONTROL
1-888-891-8332 ENVIRONMENTAL FIELD OFFICE - Columbia

NPDES Permitted Municipal/Sanitary Outfall:

TREATED MUNICIPAL/SANITARY WASTEWATER
--- STP
contact phone number
NPDES Permit NO. TN00-----
TENNESSEE DIVISION OF WATER POLLUTION CONTROL
1-888-891-8332 ENVIRONMENTAL FIELD OFFICE - Columbia

No later than sixty (60) days from the effective date of this permit, the permittee shall have the above sign(s) on display in the location specified.

3.7. ANTIDEGRADATION

Pursuant to the Rules of the Tennessee Department of Environment and Conservation, Chapter 1200-4-3-.06, titled “Tennessee Antidegradation Statement,” and in consideration of the department’s directive in attaining the greatest degree of effluent reduction achievable in municipal, industrial, and other wastes, the permittee shall further be required, pursuant to the terms and conditions of this permit, to comply with the effluent limitations and schedules of compliance required to implement applicable water quality standards, to comply with a State Water Quality Plan or other state or federal laws or regulations, or where practicable, to comply with a standard permitting no discharge of pollutants.

The pump/lift station inspection language is for permittees in Johnson City:

3.8. PUMP/LIFT STATION INSPECTION

All pump/lift stations > or = 100 gpm must be inspected five (5) days a week. In populated areas, all stations < 300 gpm may alternately be equipped with alarms, lights and or horns. In populated areas, all stations > or = 300 gpm may alternately be equipped with true remote sensing telemetry systems. All stations < 100 gpm must be inspected as necessary to ensure proper operation. The inspector shall note the date, time and inspector initials in a bound log notebook.

4.0. DEFINITIONS AND ACRONYMS

4.1. DEFINITIONS

A "***bypass***" is defined as the intentional diversion of waste streams from any portion of a treatment facility.

A "***calendar day***" is defined as the 24-hour period from midnight to midnight or any other 24-hour period that reasonably approximates the midnight to midnight time period.

A "***composite sample***" is a combination of not less than 8 influent or effluent portions, of at least 100 ml, collected over a 24-hour period. Under certain circumstances a lesser time period may be allowed, but in no case, less than 8 hours.

The "***daily maximum concentration***" is a limitation on the average concentration in units of mass per volume (e.g. milligrams per liter), of the discharge during any calendar day. When a proportional-to-flow composite sampling device is used, the daily concentration is the concentration of that 24-hour composite; when other sampling means are used, the daily concentration is the arithmetic mean of the concentrations of equal volume samples collected during any calendar day or sampling period.

"***Degradation***" means the alteration of the properties of waters by the addition of pollutants or removal of habitat. Alterations not resulting in the condition of pollution that are of a temporary nature or those alterations having de minimus impact (not measurable or less than 5 percent loss of assimilative capacity) will not be considered degradation. Degradation will not be considered de minimus if a substantial loss (more than 50 percent) of assimilative capacity has already occurred.

"***Discharge***" or "discharge of a pollutant" refers to the addition of pollutants to waters from a source.

A "***dry weather overflow***" is a type of sanitary sewer overflow and is defined as one day or any portion of a day in which unpermitted discharge of wastewater from the collection or treatment system other than through the permitted outfall occurs and is not directly related to a rainfall event. Discharges from more than one point within a 24-hour period shall be counted as separate overflows.

An "***ecoregion***" is a relatively homogeneous area defined by similarity of climate, landform, soil, potential natural vegetation, hydrology, or other ecologically relevant variables.

The "***geometric mean***" of any set of values is the nth root of the product of the individual values where "n" is equal to the number of individual values. The geometric mean is equivalent to the antilog of the arithmetic mean of the logarithms of the individual values. For the purposes of calculating the geometric mean, values of zero (0) shall be considered to be one (1).

A "***grab sample***" is a single influent or effluent sample collected at a particular time.

The "***instantaneous maximum concentration***" is a limitation on the concentration, in milligrams per liter, of any pollutant contained in the wastewater discharge determined from a grab sample taken from the discharge at any point in time.

The "***instantaneous minimum concentration***" is the minimum allowable concentration, in milligrams per liter, of a pollutant parameter contained in the wastewater discharge determined from a grab sample taken from the discharge at any point in time.

The "***monthly average amount***", shall be determined by the summation of all the measured daily discharges by weight divided by the number of days during the calendar month when the measurements were made.

The "***monthly average concentration***", other than for *E. Coli* bacteria, is the arithmetic mean of all the composite or grab samples collected in a one-calendar month period.

A "***one week period***" (or "***calendar-week***") is defined as the period from Sunday through Saturday. For reporting purposes, a calendar week that contains a change of month shall be considered part of the latter month.

"***Pollutant***" means sewage, industrial wastes, or other wastes.

A "***quarter***" is defined as any one of the following three-month periods: January 1 through March 31, April 1 through June 30, July 1 through September 30, and/or October 1 through December 31.

A "***rainfall event***" is defined as any occurrence of rain, preceded by 10 hours without precipitation that results in an accumulation of 0.01 inches or more. Instances of rainfall occurring within 10 hours of each other will be considered a single rainfall event.

A "***rationale***" (or "fact sheet") is a document that is prepared when drafting an NPDES permit or permit action. It provides the technical, regulatory and administrative basis for an agency's permit decision.

A "***reference site***" means least impacted waters within an ecoregion that have been monitored to establish a baseline to which alterations of other waters can be compared.

A "***reference condition***" is a parameter-specific set of data from regional reference sites that establish the statistical range of values for that particular substance at least-impacted streams.

A **"*sanitary sewer overflow (SSO*)"** is defined as an unpermitted discharge of wastewater from the collection or treatment system other than through the permitted outfall.

"***Sewage***" means water-carried waste or discharges from human beings or animals, from residences, public or private buildings, or industrial establishments, or boats, together with such other wastes and ground, surface, storm, or other water as may be present.

"***Severe property damage***" when used to consider the allowance of a bypass or SSO means substantial physical damage to property, damage to the treatment facilities which causes them to become inoperable, or substantial and permanent loss of natural resources which can reasonably be expected to occur in the absence of a bypass or SSO. Severe property damage does not mean economic loss caused by delays in production.

"***Sewerage system***" means the conduits, sewers, and all devices and appurtenances by means of which sewage and other waste is collected, pumped, treated, or disposed.

A "***subecoregion***" is a smaller, more homogenous area that has been delineated within an ecoregion.

"***Upset***" means an exceptional incident in which there is unintentional and temporary noncompliance with technology-based effluent limitations because of factors beyond the reasonable control of the permittee. An upset does not include noncompliance to the extent caused by operational error, improperly designed treatment facilities, inadequate treatment facilities, lack of preventive maintenance, or careless or improper operation.

The term, "***washout***" is applicable to activated sludge plants and is defined as loss of mixed liquor suspended solids (MLSS) of 30.00% or more from the aeration basin(s).

"***Waters***" means any and all water, public or private, on or beneath the surface of the ground, which are contained within, flow through, or border upon Tennessee or any portion thereof except those bodies of water confined to and retained within the limits of private property in single ownership which do not combine or effect a junction with natural surface or underground waters.

The "***weekly average amount***", shall be determined by the summation of all the measured daily discharges by weight divided by the number of days during the calendar week when the measurements were made.

The "***weekly average concentration***", is the arithmetic mean of all the composite samples collected in a one-week period. The permittee must report the highest weekly average in the one-month period.

4.2. ACRONYMNS AND ABBREVIATIONS

1Q10 – 1-day minimum, 10-year recurrence interval

30Q20 – 30-day minimum, 20-year recurrence interval

7Q10 – 7-day minimum, 10-year recurrence interval

BAT – best available technology economically achievable

BCT – best conventional pollutant control technology

BDL – below detection level

BOD_5 – five day biochemical oxygen demand

BPT – best practicable control technology currently available

$CBOD_5$ – five day carbonaceous biochemical oxygen demand

CFR – code of federal regulations

CFS – cubic feet per second

CFU – colony forming units

CIU – categorical industrial user

CSO – combined sewer overflow

DMR – discharge monitoring report

D.O. – dissolved oxygen

E. coli – *Escherichia coli*

EFO – environmental field office

LB(lb) - pound

IC_{25} – inhibition concentration causing 25% reduction in survival, reproduction and growth of the test organisms

IU – industrial user

IWS – industrial waste survey

LC_{50} – acute test causing 50% lethality

MDL – method detection level

MGD – million gallons per day

MG/L(mg/l) – milligrams per liter

ML – minimum level of quantification

ml – milliliter

MLSS – mixed liquor suspended solids

NODI – no discharge

NOEC – no observed effect concentration

NPDES – national pollutant discharge elimination system

PL – permit limit

POTW – publicly owned treatment works

RDL – required detection limit

SIU – significant industrial user

SSO – sanitary sewer overflow

STP – sewage treatment plant

TCA – Tennessee code annotated

TDEC – Tennessee Department of Environment and Conservation

TIE/TRE – toxicity identification evaluation/toxicity reduction evaluation

TMDL – total maximum daily load

TRC – total residual chlorine

TSS – total suspended solids

WQBEL – water quality based effluent limit

RATIONALE

--- STP
NPDES PERMIT No. TN00-----
DATE: 4/7/06
Permit Writer: Maybelle T. Sparks

1. FACILITY INFORMATION

--- STP
Contact person - title
---, --- County, Tennessee
Contact phone number
Treatment Plant Average Design Flow: 2 MGD
Percentage Industrial Flow: 0%
Treatment Description: Activated sludge plant with chlorination and dechlorination

2. RECEIVING STREAM INFORMATION

Receiving stream Mile --
Watershed Group: Duck-Lower
Hydrocode: 6040003
Low Flow: 7Q10 = 0.052 MGD (0.08 CFS)
Low Flow Reference:
USGS Water-Resource Investigation Report 95-4293
Station #03532200
Tier Designation: Not evaluated at this time.
Stream Classification Categories:

Domestic Wtr Supply	Industrial	Fish & Aquatic	Recreation
		X	X
Livestock Wtr & Wlife	Irrigation	Navigation	
X	X		

3. CURRENT PERMIT STATUS

Permit Type:	Municipal
Classification:	Major/Minor
Issuance Date:	3/31/2004
Expiration Date:	3/31/2008
Effective Date:	5/1/2004

The EPA Administrator, Lisa P. Jackson, signed the following final rule on April 17, 2012 and EPA is submitting it for publication in the *Federal Register* (FR). While we have taken steps to ensure the accuracy of this Internet version of the rule, it is not the official version of the rule. Please refer to the official version in a forthcoming FR publication, which will appear on the Government Printing Office's FDsys website (http://www.gpo.gov/fdsys/search/home.action) and on Regulations.gov (http://www.regulations.gov) in Docket No. EPA-HQ-OW-2010-0192. Once the official version of this document is published in the FR, this version will be removed from the Internet and replaced with a link to the official version.

Table IA–List of Approved Biological Methods for Wastewater and Sewage Sludge

Parameter and units	Method[1]	EPA	Standard Methods	AOAC, ASTM, USGS	Other
Bacteria:					
1. Coliform (fecal), number per 100 mL or number per gram dry weight	Most Probable Number (MPN),5 tube, 3 dilution, or	p. 132[3] 1680[11,15] 1681[11,20]	9221 C E–2006		
	Membrane filter (MF)[2], single step	p. 124[3]	9222 D–1997	B–0050–85[4]	
2. Coliform (fecal) in presence of chlorine, number per 100 mL	MPN, 5 tube, 3 dilution, or	p. 132[3]	9221 C E–2006		
	MF[2], single step[5]	p. 124[3]	9222 D–1997		
3. Coliform (total), number per 100 mL	MPN, 5 tube, 3 dilution, or	p. 114[3]	9221 B–2006		
	MF[2], single step or two step	p. 108[3]	9222 B–1997	B–0025–85[4]	
4. Coliform (total), in presence of chlorine, number per 100 mL	MPN, 5 tube, 3 dilution, or	p. 114[3]	9221 B–2006		
	MF[2] with enrichment[5]	p. 111[3]	9222 (B+B.5c)−1997		
5. E. coli, number per 100 mL[21]	MPN[6,8,16] multiple tube, or		9221B.1-2006/9221F-2006[12,14]		
	multiple tube/multiple well, or		9223 B–2004[13]	991.15[10]	Colilert®[13,18] Colilert-18®[13,17, 18]
	MF[2,6,7,8] single step	1603[22]			mColiBlue-24®[19]
6. Fecal streptococci, number per 100 mL	MPN, 5 tube 3 dilution, or	p. 139[3]	9230 B–2007		
	MF[2], or	p. 136[3]	9230 C–2007	B–0055–85[4]	
	Plate count	p. 143[3]			
7. Enterococci, number per 100 mL[22]	MPN[6,8], multiple tube/multiple well, or			D6503–99[9]	Enterolert®[13,24]
	MF[2,6,7,8]single step or	1600[25]	9230 C-2007		
	Plate count	p. 143[3]			
8. Salmonella, number per gram dry weight[11]	MPN multiple tube	1682[23]			
Aquatic Toxicity:					
9. Toxicity, acute, fresh water organisms, LC_{50}, percent effluent	Ceriodaphnia dubia acute	2002.0[26]			
	Daphnia puplex and Daphnia magna acute	2021.0[26]			
	Fathead Minnow, Pimephales promelas, and Bannerfin shiner, Cyprinella leedsi, acute	2000.0[26]			

Parameter and units	Method[1]	EPA	Standard Methods	AOAC, ASTM, USGS	Other
	Rainbow Trout, Oncorhynchus mykiss, and brook trout, Salvelinus fontinalis, acute	2019.0[26]			
10. Toxicity, acute, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, LC$_{50}$, percent effluent	Mysid, Mysidopsis bahia, acute	2007.0[26]			
	Sheepshead Minnow, Cyprinodon *variegatus*, acute	2004.0[26]			
	Silverside, Menidia beryllina, Menidia menidia, and Menidia peninsulae, acute	2006.0[26]			
11. Toxicity, chronic, fresh water organisms, NOEC or IC$_{25}$, percent effluent	Fathead minnow, Pimephales promelas, larval survival and growth	1000.0[27]			
	Fathead minnow, Pimephales promelas, embryo-larval survival and teratogenicity	1001.0[27]			
	Daphnia, Ceriodaphnia dubia, survival and reproduction	1002.0[27]			
	Green alga, Selenastrum capricornutum, growth	1003.0[27]			
12. Toxicity, chronic, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, NOEC or IC$_{25}$, percent effluent	Sheepshead minnow, Cyprinodon variegatus, larval survival and growth	1004.0[28]			
	Sheepshead minnow, Cyprinodon variegatus, embryo-larval survival and teratogenicity	1005.0[28]			
	Inland silverside, Menidia beryllina, larval survival and growth	1006.0[28]			
	Mysid, Mysidopsis bahia, survival, growth, and fecundity	1007.0[28]			
	Sea urchin, Arbacia punctulata, fertilization	1008.0[28]			

[1] The method must be specified when results are reported.

[2] A 0.45-µm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth.

[3] Microbiological Methods for Monitoring the Environment, Water, and Wastes, EPA/600/8–78/017. 1978. US EPA.

[4] U.S. Geological Survey Techniques of Water-Resource Investigations, Book 5, Laboratory Analysis, Chapter A4, Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples. 1989. USGS..

[5] Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies.

[6] Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample.

[7] When the MF method has been used previously to test waters with high turbidity, large numbers of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results.

[8] To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current Standard Methods for the Examination of Water and Wastewater or EPA alternate test procedure (ATP) guidelines.

[9] Annual Book of ASTM Standards–Water and Environmental Technology, Section 11.02. 2000, 1999, 1996. ASTM International.

[10] Official Methods of Analysis of AOAC International. 16th Edition, 4th Revision, 1998. AOAC International

[11] Recommended for enumeration of target organism in sewage sludge.

[12] The multiple-tube fermentation test is used in 9221B.1-2006. Lactose broth may be used in lieu of lauryl tryptose broth (LTB), if at least 25 parallel tests are conducted between this broth and LTB using the water samples normally tested, and this comparison demonstrates that the false-positive rate and false-negative rate for total coliform using lactose broth is less than 10 percent. No requirement exists to run the completed phase on 10 percent of all total coliform-positive tubes on a seasonal basis.

[13] These tests are collectively known as defined enzyme substrate tests, where, for example, a substrate is used to detect the enzyme β-glucuronidase produced by E. coli.

[14] After prior enrichment in a presumptive medium for total coliform using 9221B.1-2006, all presumptive tubes or bottles showing any amount of gas, growth or acidity within 48 h ± 3 h of incubation shall be submitted to 9221F-2006. Commercially available EC-MUG media or EC media supplemented in the laboratory with 50 µg/mL of MUG may be used.

[15] Method 1680: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation Using Lauryl-Tryptose Broth (LTB) and EC Medium, EPA–821–R–10–003. April 2010. US EPA.

[16] Samples shall be enumerated by the multiple-tube or multiple-well procedure. Using multiple-tube procedures, employ an appropriate tube and dilution configuration of the sample as needed and report the Most Probable Number (MPN). Samples tested with Colilert® may be enumerated with the multiple-well procedures, Quanti-Tray®, Quanti-Tray®/2000, and the MPN calculated from the table provided by the manufacturer.

[17] Colilert-18® is an optimized formulation of the Colilert® for the determination of total coliforms and E. coli that provides results within 18 h of incubation at 35°C rather than the 24 h required for the Colilert® test and is recommended for marine water samples.

[18] Descriptions of the Colilert®, Colilert-18®, Quanti-Tray®, and Quanti-Tray®/2000 may be obtained from IDEXX Laboratories, Inc.

[19] A description of the mColiBlue24® test, is available from Hach Company.

[20] Method 1681: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using A–1 Medium, EPA–821–R–06–013. July 2006. US EPA.

[21] Recommended for enumeration of target organism in wastewater effluent.

[22] Method 1603: Escherichia coli (E. coli) in Water by Membrane Filtration Using Modified membrane-Thermotolerant Escherichia coli Agar (modified mTEC), EPA–821–R–09–007. December 2009. US EPA.

[23] Method 1682: Salmonella in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium, EPA–821–R–06–014. July 2006. US EPA.

[24] A description of the Enterolert® test may be obtained from IDEXX Laboratories Inc.

[25] Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI), EPA–821–R–09–016. December 2009. US EPA.

[26] Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA-821-R-02-012. Fifth Edition, October 2002. US EPA.

[27] Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA-821-R-02-013. Fourth Edition, October 2002. US EPA.

[28] Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. EPA-821-R-02-014. Third Edition, October 2002. US EPA.

TABLE IB – LIST OF APPROVED INORGANIC TEST PROCEDURES

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
1. Acidity, as $CaCO_3$, mg/L	Electrometric endpoint or phenolphthalein endpoint		2310 B-1997	D1067–06	I–1020–85[2]
2. Alkalinity, as $CaCO_3$, mg/L	Electrometric or Colorimetric titration to pH 4.5, Manual		2320 B-1997	D1067–06	973.43[3], I–1030–85[2]
	Automatic	310.2 (Rev. 1974)[1]			I–2030–85[2]
3. Aluminum–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration[36]		3111 D-1999 or 3111 E-1999		I–3051–85[2]
	AA furnace		3113 B-2004		
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4471–97[50]
	Direct Current Plasma (DCP)[36]			D4190–08	See footnote[34]
	Colorimetric (Eriochrome cyanine R)		3500–Al B-2001		
4. Ammonia (as N), mg/L	Manual distillation[6] or gas diffusion (pH > 11), followed by any of the following:	350.1, Rev. 2.0 (1993)	4500–NH_3 B-1997		973.49[3]
	Nesslerization			D1426–08 (A)	973.49[3], I–3520–85[2]
	Titration		4500–NH_3 C-1997		
	Electrode		4500–NH_3 D-1997 or E-1997	D1426–08 (B)	
	Manual phenate, salicylate, or other substituted phenols in Berthelot reaction based methods		4500–NH_3 F-1997		See footnote [60]
	Automated phenate, salicylate, or other substituted phenols in Berthelot reaction based methods	350.1[30], Rev. 2.0 (1993)	4500–NH_3 G-1997 4500-NH_3 H-1997		I–4523–85[2]
	Automated electrode				See footnote [7]

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	Ion Chromatography			D6919–09	
5. Antimony–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration[36]		3111 B-1999		
	AA furnace		3113 B-2004		
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4471–97[50]
6. Arsenic–Total,[4] mg/L	Digestion[4], followed by any of the following:	206.5 (Issued 1978)[1]			
	AA gaseous hydride		3114 B-2009 or 3114 C-2009	D2972–08 (B)	I–3062–85[2]
	AA furnace		3113 B-2004	D2972–08 (C)	I–4063–98[49]
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4020–05[70]
	Colorimetric (SDDC)		3500–As B-1997	D2972–08 (A)	I–3060–85[2]
7. Barium–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration[36]		3111 D-1999		I–3084–85[2]
	AA furnace		3113 B-2004	D4382–02(07)	
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4471–97[50]
	DCP[36]				See footnote[34]
8. Beryllium–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration		3111 D-1999 or 3111 E-1999	D3645–08 (A)	I–3095–85[2]
	AA furnace		3113 B-2004	D3645–08 (B)	

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4471–97[50]
	DCP			D4190–08	See footnote[34]
	Colorimetric (aluminon)		See footnote[61]		
9. Biochemical oxygen demand (BOD5), mg/L	Dissolved Oxygen Depletion		5210 B-2001		973.44,[3] p. 17.[9], I–1578–78[8], See footnote[10, 63]
10. Boron–Total,[37] mg/L	Colorimetric (curcumin)		4500–B B - 2000		I–3112–85[2]
	ICP/AES	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4471–97[50]
	DCP			D4190–08	See footnote [34]
11. Bromide, mg/L	Electrode			D1246–05	I–1125–85[2]
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1-1, Rev 1.0 (1997)	4110 B-2000, C-2000, D-2000	D4327–03	993.30[3]
	CIE/UV		4140 B-1997	D6508–00(05)	D6508, Rev. 2[54]
12. Cadmium–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration[36]		3111 B -1999 or 3111 C-1999	D3557–02(07) (A or B)	974.27,[3] p. 37.[9], I–3135–85[2] or I–3136–85[2]
	AA furnace		3113 B -2004	D3557–02(07) (D)	I–4138–89[51]
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–1472–85[2] or I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4471–97[50]
	DCP[36]			D4190–08	See footnote[34]

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	Voltametry[11]			D3557–02(07) (C)	
	Colorimetric (Dithizone)		3500-Cd-D-1990		
13. Calcium–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration		3111 B-1999	D511–08(B)	I–3152–85[2]
	ICP/AES	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3]
	DCP				See footnote[34]
	Titrimetric (EDTA)		3500–Ca B-1997	D511–08 (A)	
	Ion Chromatography			D6919–09	
14. Carbonaceous biochemical oxygen demand (CBOD$_5$), mg/L[12]	Dissolved Oxygen Depletion with nitrification inhibitor		5210 B-2001		See footnote[35, 63]
15. Chemical oxygen demand (COD), mg/L	Titrimetric	410.3 (Rev. 1978)[1]	5220 B-1997 or C-1997	D1252–06 (A)	973.46[3], p. 17[9,] I–3560–85[2]
	Spectrophotometric, manual or automatic	410.4, Rev. 2.0 (1993)	5220 D-1997	D1252–06 (B)	See footnotes[13, 14]. I–3561–85[2]
16. Chloride, mg/L	Titrimetric: (silver nitrate)		4500–Cl^- B-1997	D512–04 (B)	I–1183–85[2]
	(Mercuric nitrate)		4500–Cl^- C-1997	D512–04 (A)	973.51[3], I–1184–85[2]
	Colorimetric: manual				I–1187–85[2]
	Automated (Ferricyanide)		4500–Cl^- E-1997		I–2187–85[2]
	Potentiometric Titration		4500–Cl^- D-1997		
	Ion Selective Electrode			D512–04 (C)	
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1-1, Rev 1.0 (1997)	4110 B-2000 or 4110 C-2000	D4327–03	993.30[3] , I–2057–90[51]
	CIE/UV		4140 B-1997	D6508–00(05)	D6508, Rev. 2[54]
17. Chlorine–Total residual, mg/L	Amperometric direct		4500–Cl D-2000	D1253–08	
	Amperometric direct (low level)		4500–Cl E-2000		

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	Iodometric direct		4500–Cl B-2000		
	Back titration ether end–point[15]		4500–Cl C-2000		
	DPD–FAS		4500–Cl F-2000		
	Spectrophotometric, DPD		4500–Cl G-2000		
	Electrode				See footnote[16]
17A. Chlorine–Free Available, mg/L	Amperometric direct		4500–Cl D-2000	D1253–08	
	Amperometric direct (low level)		4500–Cl E-2000		
	DPD–FAS		4500–Cl F-2000		
	Spectrophotometric, DPD		4500–Cl G-2000		
18. Chromium VI dissolved, mg/L	0.45-micron Filtration followed by any of the following:				
	AA chelation–extraction		3111 C-1999		I–1232–85[2]
	Ion Chromatography	218.6, Rev. 3.3 (1994)	3500–Cr C-2009	D5257–03	993.23
	Colorimetric (Diphenyl–carbazide)		3500–Cr B-2009	D1687–02(07) (A)	I–1230–85[2]
19. Chromium–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration[36]		3111 B-1999	D1687–02(07) (B)	974.27[3], I–3236–85[2]
	AA chelation–extraction		3111 C-1999		
	AA furnace		3113 B-2004	D1687–02(07) (C)	I–3233–93[46]
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4020–05[70]
	DCP[36]			D4190–08	See footnote[34]
	Colorimetric (Diphenyl–carbazide)		3500–Cr B-2009		
20. Cobalt–Total,[4] mg/L	Digestion[4], followed by any of the following:				

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	AA direct aspiration		3111 B-1999 or 3111 C-1999	D3558–08 (A or B)	p. 37[9], I–3239–85[2]
	AA furnace		3113 B-2004	D3558–08 (C)	I–4243–89[51]
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4020–05[70]
	DCP			D4190–08	See footnote[34]
21. Color, platinum cobalt units or dominant wavelength, hue, luminance purity	Colorimetric (ADMI)				See footnote[18]
	(Platinum cobalt)		2120 B-2001		I–1250–85[2]
	Spectrophotometric				
22. Copper–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration[36]		3111 B-1999 or 3111 C-1999	D1688–07 (A or B)	974.27[3] p. 37[9], I–3270–85[2]or I–3271–85[2]
	AA furnace		3113 B-2004	D1688–07 (C)	I–4274–89[51]
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4020–05[70]
	DCP[36]			D4190–08	See footnote[34]
	Colorimetric (Neocuproine)		3500–Cu B-1999		
	(Bathocuproine)		3500–Cu C-1999		See footnote[19]
23. Cyanide–Total, mg/L	Automated UV digestion /distillation and Colorimetry				Kelada–01[55]
	Segmented Flow Injection, In-Line Ultraviolet Digestion, followed by gas diffusion amperometry			D7511-09	
	Manual distillation with $MgCl_2$, followed by any of the following:	335.4, Rev. 1.0 (1993)[57]	4500–CN^- B-1999 or C-1999	D2036–09(A), D7284-08	10–204–00–1–X[56]

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	Flow Injection, gas diffusion amperometry			D2036-09(A) D7284-08	
	Titrimetric		4500–CN$^-$ D-1999	D2036-09(A)	p. 22[9]
	Spectrophotometric, manual		4500–CN$^-$ E-1999	D2036–09(A)	I–3300–85[2]
	Semi-Automated[20]	335.4, Rev. 1.0 (1993)[57]			10–204–00–1–X[56], I–4302–85[2]
	Ion Chromatography			D2036-09(A)	
	Ion Selective Electrode		4500–CN$^-$ F-1999	D2036–09(A)	
24. Cyanide–Available, mg/L	Cyanide Amenable to Chlorination (CATC); Manual distillation with MgCl$_2$, followed by Titrimetric or Spectrophotometric		4500–CN$^-$ G-1999	D2036–09(B)	
	Flow injection and ligand exchange, followed by gas diffusion amperometry[59]			D6888–09	OIA–1677-09[44]
	Automated Distillation and Colorimetry (no UV digestion)				Kelada–01[55]
24.A Cyanide-Free, mg/L	Flow Injection, followed by gas diffusion amperometry			D7237-10	OIA–1677-09[44]
	Manual micro-diffusion and colorimetry			D4282-02	
25. Fluoride–Total, mg/L	Manual distillation[6], followed by any of the following:		4500–F$^-$ B-1997		
	Electrode, manual		4500–F$^-$ C-1997	D1179–04 (B)	
	Electrode, automated				I–4327–85[2]
	Colorimetric, (SPADNS)		4500–F$^-$ D-1997	D1179–04 (A)	
	Automated complexone		4500–F$^-$ E-1997		
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1-1, Rev 1.0 (1997)	4110 B-2000 or C-2000	D4327–03	993.30[3]
	CIE/UV		4140 B-1997	D6508–00(05)	D6508, Rev. 2[54]
26. Gold–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration		3111 B-1999		

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	AA furnace	231.2 (Issued1978)[1]	3113 B-2004		
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3]
	DCP				See footnote[34]
27. Hardness–Total, as $CaCO_3$, mg/L	Automated colorimetric	130.1 (Issued 1971)[1]			
	Titrimetric (EDTA)		2340 C-1997	D1126–02(07)	973.52B[3], I–1338–85[2]
	Ca plus Mg as their carbonates, by inductively coupled plasma or AA direct aspiration. (See Parameters 13 and 33).		2340 B-1997		
28. Hydrogen ion (pH), pH units	Electrometric measurement		4500–H^+ B-2000	D1293–99 (A or B)	973.41[3], I–1586–85[2]
	Automated electrode	150.2 (Dec. 1982)[1]			See footnote[21], I–2587–85[2]
29. Iridium–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration		3111 B-1999		
	AA furnace	235.2 (Issued 1978)[1]			
	ICP/MS		3125 B-2009		
30. Iron–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration[36]		3111 B-1999 or 3111 C-1999	D1068–05 (A or B)	974.27[3], I–3381–85[2]
	AA furnace		3113 B-2004	D1068–05 (C)	
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3]
	DCP[36]			D4190–08	See footnote[34]
	Colorimetric (Phenanthroline)		3500–Fe-1997	D1068–05 (D)	See footnote[22]
31. Kjeldahl Nitrogen[5]–Total, (as N), mg/L	Manual digestion[20] and distillation or gas diffusion, followed by any of the following:		4500–N_{org} B-1997 or C-1997 and 4500–NH_3 B-1997	D3590–02(06) (A)	I–4515–91[45]

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	Titration		4500–NH$_3$ C-1997		973.48[3]
	Nesslerization			D1426–08 (A)	
	Electrode		4500–NH$_3$ D-1997 or E-1997	D1426–08 (B)	
	Semi-automated phenate	350.1 Rev 2.0 1993	4500–NH$_3$ G-1997 4500–NH$_3$ H-1997		
	Manual phenate, salicylate, or other substituted phenols in Berthelot reaction based methods		4500–NH$_3$ F-1997		See footnote[60]
	Automated Methods for TKN that do not require manual distillation				
	Automated phenate, salicylate, or other substituted phenols in Berthelot reaction based methods colorimetric (auto digestion and distillation)	351.1 (Rev. 1978)[1]			I–4551–78[8]
	Semi-automated block digestor colorimetric (distillation not required)	351.2, Rev. 2.0 (1993)	4500–N$_{org}$ D-1997	D3590–02(06) (B)	I–4515–91[45]
	Block digester, followed by Auto distillation and Titration				See footnote[39]
	Block digester, followed by Auto distillation and Nesslerization				See footnote[40]
	Block Digester, followed by Flow injection gas diffusion (distillation not required)				See footnote[41]
32. Lead–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration[36]		3111 B-1999 or 3111 C-1999	D3559–08 (A or B)	974.27[3], I–3399–85[2]
	AA furnace		3113 B-2004	D3559–08 (D)	I–4403–89[51]
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4471–97[50]
	DCP[36]			D4190–08	See footnote[34]
	Voltametry[11]			D3559–08 (C)	
	Colorimetric (Dithizone)		3500–Pb B-1997		
33. Magnesium–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration		3111 B-1999	D511–08 (B)	974.27[3], I–3447–85[2]
	ICP/AES	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3]
	DCP				See footnote[34]
	Gravimetric				
	Ion Chromatography			D6919–09	
34. Manganese–Total,[4] mg/L	Digestion[4] followed by any of the following:				
	AA direct aspiration[36]		3111 B-1999	D858–07 (A or B)	974.27[3], I–3454–85[2]
	AA furnace		3113 B-2004	D858–07 (C)	
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4471–97[50]
	DCP[36]			D4190–08	See footnote[34]
	Colorimetric (Persulfate)		3500–Mn B-1999		920.203[3]
	(Periodate)				See footnote[23]
35. Mercury–Total[4], mg/L	Cold vapor, Manual	245.1, Rev. 3.0 (1994)	3112 B-2009	D3223–02(07)	977.22[3], I–3462–85[2]
	Cold vapor, Automated	245.2 (Issued 1974)[1]			
	Cold vapor atomic fluorescence spectrometry (CVAFS)	245.7 Rev. 2.0 (2005)[17]			I-4464-01[71]
	Purge and Trap CVAFS	1631E[43]			
36. Molybdenum–Total[4], mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration		3111 D-1999		I–3490–85[2]
	AA furnace		3113 B-2004		I–3492–96[47]

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4471–97[50]
	DCP				See footnote[34]
37. Nickel–Total,[4] mg/L	Digestion[4] followed by any of the following:				
	AA direct aspiration[36]		3111 B-1999 or 3111 C-1999	D1886–08 (A or B)	I–3499–85[2]
	AA furnace		3113 B-2004	D1886–08 (C)	I–4503–89[51]
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4020–05[70]
	DCP[36]			D4190–08	See footnote[34]
38. Nitrate (as N), mg/L	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1-1, Rev 1.0 (1997)	4110 B-2000 or C-2000	D4327–03	993.30[3]
	CIE/UV		4140 B-1997	D6508–00(05)	D6508, Rev. 2[54]
	Ion Selective Electrode		4500–NO_3^- D-2000		
	Colorimetric (Brucine sulfate)	352.1 (Issued 1971)[1]			973.50[3], 419D[1,7], p. 28[9]
	Nitrate-nitrite N minus Nitrite N (See parameters 39 and 40).				See footnote[62]
39. Nitrate-nitrite (as N), mg/L	Cadmium reduction, Manual		4500–NO_3^- E-2000	D3867–04 (B)	
	Cadmium reduction, Automated	353.2, Rev. 2.0 (1993)	4500–NO_3^- F-2000	D3867–04 (A)	I–2545–90[51]
	Automated hydrazine		4500–NO_3^- H-2000		
	Reduction/Colorimetric				See footnote[62]
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1-1, Rev 1.0 (1997)	4110 B-2000 or C-2000	D4327–03	993.30[3]
	CIE/UV		4140 B-1997	D6508–00(05)	D6508, Rev. 2[54]

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
40. Nitrite (as N), mg/L	Spectrophotometric: Manual		4500–NO_2^- B-2000		See footnote[25]
	Automated (Diazotization)				I–4540–85[2], See footnote[62]
	Automated (*bypass cadmium reduction)	353.2, Rev. 2.0 (1993)	4500–NO_3^- F-2000	D3867–04 (A)	I–4545–85[2]
	Manual (*bypass cadmium reduction)		4500–NO_3^- E-2000	D3867–04 (B)	
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1-1, Rev 1.0 (1997)	4110 B-2000 or C-2000	D4327–03	993.30[3]
	CIE/UV		4140 B-1997	D6508–00(05)	D6508, Rev.2[54]
41. Oil and grease–Total recoverable, mg/L	Hexane extractable material (HEM): n–Hexane extraction and gravimetry	1664 Rev. A; 1664 Rev. B[42]	5520 B-2001[38]		
	Silica gel treated HEM (SGT–HEM): Silica gel treatment and gravimetry.	1664 Rev. A; 1664 Rev. B[42]	5520 B-2001[38] and 5520 F-2001[38]		
42. Organic carbon–Total (TOC), mg/L	Combustion		5310 B-2000	D7573–09	973.47[3], p. 14[24]
	Heated persulfate or UV persulfate oxidation		5310 C 2000 5310 D 2000	D4839–03	973.47[3,], p. 14[24]
43. Organic nitrogen (as N), mg/L	Total Kjeldahl N (Parameter 31) minus ammonia N (Parameter 4)				
44. Ortho-phosphate (as P), mg/L	Ascorbic acid method:				
	Automated	365.1, Rev. 2.0 (1993)	4500-P F-1999 or G-1999		973.56[3], I–4601–85[2]
	Manual single reagent		4500-P E-1999	D515–88(A)	973.55[3]
	Manual two reagent	365.3 (Issued 1978)[1]			
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1-1, Rev 1.0 (1997)	4110 B-2000 or C-2000	D4327–03	993.30[3]
	CIE/UV		4140 B-1997	D6508–00(05)	D6508, Rev. 2[54]
45. Osmium–Total[4], mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration,		3111 D-1999		
	AA furnace	252.2 (Issued 1978)[1]			

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
46. Oxygen, dissolved, mg/L	Winkler (Azide modification)		4500–O B-2001, C-2001, D-2001, E-2001, F-2001	D888–09 (A)	973.45B[3], I–1575–78[8]
	Electrode		4500–O G-2001	D888–09 (B)	I–1576–78[8]
	Luminescence Based Sensor			D888–09 (C)	See footnote[63] See footnote[64]
47. Palladium–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration		3111 B-1999		
	AA furnace	253.2[1](Issued 1978)			
	ICP/MS		3125 B-2009		
	DCP				See footnote[34]
48. Phenols, mg/L	Manual distillation[26], followed by any of the following:	420.1[1](Rev. 1978)	5530 B-2005	D1783–01	
	Colorimetric (4AAP) manual	420.1[1](Rev. 1978)	5530 D-2005[27]	D1783–01 (A or B)	
	Automated colorimetric (4AAP)	420.4 Rev. 1.0 (1993)			
49. Phosphorus (elemental), mg/L	Gas–liquid chromatography				See footnote[28]
50. Phosphorus–Total, mg/L	Digestion[20], followed by any of the following:		4500-P B(5)-1999		973.55[3]
	Manual	365.3[1](Issued 1978)	4500-P E-1999	D515–88 (A)	
	Automated ascorbic acid reduction	365.1 Rev. 2.0 (1993)	4500-P F-1999, G-1999, H-1999		973.56[3], I–4600–85[2]
	ICP/AES[4, 36]	200.7, Rev. 4.4 (1994)	3120 B-1999		I–4471–97[50]
	Semi–automated block digestor (TKP digestion)	365.4[1] (Issued 1974)		D515–88 (B)	I–4610–91[48]
51. Platinum–Total,[4] mg/L	Digestion[4] followed by any of the following:				
	AA direct aspiration		3111 B-1999		
	AA furnace	255.2 (Issued 1978)[1]			
	ICP/MS		3125 B-2009		
	DCP				See footnote[34]
52. Potassium–Total,[4] mg/L	Digestion[4], followed by any of the following:				

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	AA direct aspiration		3111 B-1999		973.53[3], I–3630–85[2]
	ICP/AES	200.7, Rev. 4.4 (1994)	3120 B-1999		
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3]
	Flame photometric		3500–K B-1997		
	Electrode		3500-K C-1997		
	Ion Chromatography			D6919–09	
53. Residue–Total, mg/L	Gravimetric, 103–105°		2540 B-1997		I–3750–85[2]
54. Residue–filterable, mg/L	Gravimetric, 180°		2540 C-1997	D5907–03	I–1750–85[2]
55. Residue–non–filterable (TSS), mg/L	Gravimetric, 103–105° post washing of residue		2540 D-1997	D5907–03	I–3765–85[2]
56. Residue–settleable, mg/L	Volumetric, (Imhoff cone), or gravimetric		2540 F-1997		
57. Residue–Volatile, mg/L	Gravimetric, 550°	160.4 (Issued 1971)[1]	2540-E-1997		I–3753–85[2]
58. Rhodium–Total,[4] mg/L	Digestion[4] followed by any of the following:				
	AA direct aspiration, or		3111 B-1999		
	AA furnace	265.2 (Issued 1978)[1]			
	ICP/MS		3125 B-2009		
59. Ruthenium–Total,[4] mg/L	Digestion[4] followed by any of the following:				
	AA direct aspiration, or		3111 B-1999		
	AA furnace	267.2[1]			
	ICP/MS		3125 B-2009		
60. Selenium–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA furnace		3113 B-2004	D3859–08 (B)	I–4668–98[49]
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4020–05[70]

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	AA gaseous hydride		3114 B-2009,or 3111 C-2009	D3859–08 (A)	I–3667–85[2]
61. Silica–Dissolved,[37] mg/L	0.45-micron filtration followed by any of the following:				
	Colorimetric, Manual		4500–SiO_2 C-1997	D859–05	I–1700–85[2]
	Automated (Molybdosilicate)		4500–SiO_2 E-1997 or F-1997		I–2700–85[2]
	ICP/AES	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3]
62. Silver–Total,[4, 31] mg/L	Digestion[4, 29], followed by any of the following:				
	AA direct aspiration		3111 B-1999 or 3111 C-1999		974.27[3], p. 37[9], I–3720–85[2]
	AA furnace		3113 B -2004		I–4724–89[51]
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4471–97[50]
	DCP				See footnote[34]
63. Sodium–Total,[4] mg/L	Digestion[4,], followed by any of the following:				
	AA direct aspiration		3111 B-1999		973.54[3], I–3735–85[2]
	ICP/AES	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999		I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3]
	DCP				See footnote[34]
	Flame photometric		3500–Na B-1997		
	Ion Chromatography			D6919–09	
64. Specific conductance, micromhos/cm at 25°C	Wheatstone bridge	120.1[1](Rev. 1982)	2510 B-1997	D1125–95(99) (A)	973.40[3], I–2781–85[2]

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
65. Sulfate (as SO_4), mg/L	Automated colorimetric	375.2, Rev. 2.0 (1993)	4500-SO_4^{2-} F-1997 or G-1997		
	Gravimetric		4500-SO_4^{2-} C-1997 or D-1997		925.54[3]
	Turbidimetric		4500-SO_4^{2-} E-1997	D516–07	
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1-1, Rev 1.0 (1997)	4110 B-2000 or C-2000	D4327–03	993.30[3], I–4020–05[70]
	CIE/UV		4140 B-1997	D6508–00(05)	D6508, Rev. 2[54]
66. Sulfide (as S), mg/L	Sample Pretreatment		4500–$S^{2–}$ B, C-2000		
	Titrimetric (iodine)		4500–$S^{2–}$F-2000		I–3840–85[2]
	Colorimetric (methylene blue)		4500–$S^{2–}$D-2000		
	Ion Selective Electrode		4500–$S^{2–}$G-2000	D4658–08	
67. Sulfite (as SO_3), mg/L	Titrimetric (iodine-iodate)		4500–$SO_3^{2–}$ B-2000		
68. Surfactants, mg/L	Colorimetric (methylene blue)		5540 C-2000	D2330–02	
69. Temperature, °C	Thermometric		2550 B-2000		See footnote[32]
70. Thallium–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration		3111 B-1999		
	AA furnace	279.2[1](Issued 1978)	3113 B-2004		
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES	200.7, Rev. 4.4 (1994);); 200.5 Rev. 4.2 (2003)[68]	3120 B-1999	D1976–07	
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4471–97[50]
71. Tin–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration		3111 B-1999		I–3850–78[8]
	AA furnace		3113 B-2004		
	STGFAA	200.9, Rev. 2.2 (1994)			

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
	ICP/AES	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)			
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3]
72. Titanium–Total,[4] mg/L	Digestion[4] followed by any of the following:				
	AA direct aspiration		3111 D-1999		
	AA furnace	283.2[1](Issued 1978)			
	ICP/AES	200.7, Rev. 4.4 (1994)			
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3]
	DCP				See footnote[34]
73. Turbidity, NTU[53]	Nephelometric	180.1, Rev. 2.0 (1993)	2130 B-2001	D1889–00	I–3860–85[2] See footnote[65] See footnote[66] See footnote[67]
74. Vanadium–Total,[4] mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration		3111 D-1999		
	AA furnace		3113 B-2004	D3373–03(07)	
	ICP/AES	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4020–05[70]
	DCP			D4190–08	See footnote[34]
	Colorimetric (Gallic Acid)		3500–V B-1997		
75. Zinc–Total[4], mg/L	Digestion[4], followed by any of the following:				
	AA direct aspiration[36]		3111 B-1999 or 3111 C-1999	D1691–02(07) (A or B)	974.27[3], p. 37[9], I–3900–85[2]
	AA furnace	289.2[1](Issued 1978)			
	ICP/AES[36]	200.5, Rev 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-1999	D1976–07	I–4471–97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2009	D5673–05	993.14[3], I–4020–05[70]
	DCP[36]			D4190–08	See footnote[34]
	Colorimetric (Zincon)		3500 Zn B-1997		See footnote[33]

Parameter	Methodology[58]	EPA[52]	Standard Methods	ASTM	USGS/AOAC/Other
76. Acid Mine Drainage		1627[69]			

Table IB Notes:

[1] Methods for Chemical Analysis of Water and Wastes,EPA–600/4–79–020. Revised March 1983 and 1979, where applicable. US EPA.

[2] Methods for Analysis of Inorganic Substances in Water and Fluvial Sediments, Techniques of Water-Resource Investigations of the U.S. Geological Survey, Book 5, Chapter A1., unless otherwise stated. 1989. USGS.

[3] Official Methods of Analysis of the Association of Official Analytical Chemists, Methods Manual, Sixteenth Edition, 4th Revision, 1998. AOAC International.

[4] For the determination of total metals (which are equivalent to total recoverable metals) the sample is not filtered before processing. A digestion procedure is required to solubilize analytes in suspended material and to break down organic-metal complexes (to convert the analyte to a detectable form for colorimetric analysis). For non–platform graphite furnace atomic absorption determinations a digestion using nitric acid (as specified in Section 4.1.3 of Methods for the Chemical Analysis of Water and Wastes) is required prior to analysis. The procedure used should subject the sample to gentle, acid refluxing and at no time should the sample be taken to dryness. For direct aspiration flame atomic absorption determinations (FLAA) a combination acid (nitric and hydrochloric acids) digestion is preferred prior to analysis. The approved total recoverable digestion is described as Method 200.2 in Supplement I of "Methods for the Determination of Metals in Environmental Samples" EPA/600R–94/111, May, 1994, and is reproduced in EPA Methods 200.7, 200.8, and 200.9 from the same Supplement. However, when using the gaseous hydride technique or for the determination of certain elements such as antimony, arsenic, selenium, silver, and tin by non–EPA graphite furnace atomic absorption methods, mercury by cold vapor atomic absorption, the noble metals and titanium by FLAA, a specific or modified sample digestion procedure may be required and in all cases the referenced method write–up should be consulted for specific instruction and/or cautions. For analyses using inductively coupled plasma-atomic emission spectrometry (ICP–AES), the direct current plasma (DCP) technique or the EPA spectrochemical techniques (platform furnace AA, ICP–AES, and ICP–MS) use EPA Method 200.2 or an approved alternate procedure (e.g., CEM microwave digestion, which may be used with certain analytes as indicated in Table IB); the total recoverable digestion procedures in EPA Methods 200.7, 200.8, and 200.9 may be used for those respective methods. Regardless of the digestion procedure, the results of the analysis after digestion procedure are reported as "total" metals.

[5] Copper sulfate or other catalysts that have been found suitable may be used in place of mercuric sulfate.

[6] Manual distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary: however, manual distillation will be required to resolve any controversies. In general, the analytical method should be consulted regarding the need for distillation. If the method is not clear, the laboratory may compare a minimum of 9 different sample matrices to evaluate the need for distillation. For each matrix, a matrix spike and matrix spike duplicate are analyzed both with and without the distillation step. (A total of 36 samples, assuming 9 matrices). If results are comparable, the laboratory may dispense with the distillation step for future analysis. Comparable is defined as < 20% RPD for all tested matrices). Alternatively the two populations of spike recovery percentages may be compared using a recognized statistical test.

[7] Industrial Method Number 379–75 WE Ammonia, Automated Electrode Method, Technicon Auto Analyzer II. February 19, 1976. Bran & Luebbe Analyzing Technologies Inc.

[8] The approved method is that cited in Methods for Determination of Inorganic Substances in Water and Fluvial Sediments, Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 5, Chapter A1. 1979. USGS.

[9] American National Standard on Photographic Processing Effluents. April 2, 1975. .American National Standards Institute.

[10] In-Situ Method 1003-8-2009, Biochemical Oxygen Demand (BOD) Measurement by Optical Probe. 2009. In-Situ Incorporated.

[11] The use of normal and differential pulse voltage ramps to increase sensitivity and resolution is acceptable.

Table II - Required Containers, Preservation Techniques, and Holding Times

Parameter Number/Name	Container[1]	Preservation[2, 3]	Maximum Holding Time[4]
Table IA - Bacterial Tests:			
1-5. Coliform, total, fecal, and E. coli	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$[5]	8 hours[22,23]
6. Fecal streptococci	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$[5]	8 hours[22]
7. Enterococci	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$[5]	8 hours[22]
8. Salmonella	PA, G	Cool, <10 °C, 0.0008% $Na_2S_2O_3$[5]	8 hours[22]
Table IA - Aquatic Toxicity Tests:			
9-12. Toxicity, acute and chronic	P, FP, G	Cool, ≤6 °C[16]	36 hours
Table IB - Inorganic Tests:			
1. Acidity	P, FP, G	Cool, ≤6 °C[18]	14 days
2. Alkalinity	P, FP, G	Cool, ≤6 °C[18]	14 days
4. Ammonia	P, FP, G	Cool, ≤6 °C[18], H_2SO_4 to pH<2	28 days
9. Biochemical oxygen demand	P, FP, G	Cool, ≤6 °C[18]	48 hours
10. Boron	P, FP, or Quartz	HNO_3 to pH<2	6 months
11. Bromide	P, FP, G	None required	28 days
14. Biochemical oxygen demand, carbonaceous	P, FP G	Cool, ≤6 °C[18]	48 hours
15. Chemical oxygen demand	P, FP, G	Cool, ≤6 °C[18], H_2SO_4 to pH<2	28 days
16. Chloride	P, FP, G	None required	28 days
17. Chlorine, total residual	P, G	None required	Analyze within 15 minutes
21. Color	P, FP, G	Cool, ≤6 °C[18]	48 hours
23-24. Cyanide, total or available (or CATC) and free	P, FP, G	Cool, ≤6 °C[18], NaOH to pH>10[5, 6], reducing agent if oxidizer present	14 days
25. Fluoride	P	None required	28 days
27. Hardness	P, FP, G	HNO_3 or H_2SO_4 to pH<2	6 months
28. Hydrogen ion (pH)	P, FP, G	None required	Analyze within 15 minutes
31, 43. Kjeldahl and organic N	P, FP, G	Cool, ≤6 °C[18], H_2SO_4 to pH<2	28 days
Table IB - Metals:[7]			
18. Chromium VI	P, FP, G	Cool, ≤6 °C[18], pH = 9.3 - 9.7[20]	28 days
35. Mercury (CVAA)	P, FP, G	HNO_3 to pH<2	28 days
35. Mercury (CVAFS)	FP, G; and FP-lined cap[17]	5 mL/L 12N HCl or 5 mL/L BrCl[17]	90 days[17]
3, 5-8, 12, 13, 19, 20, 22, 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	P, FP, G	HNO_3 to pH<2, or at least 24 hours prior to analysis[19]	6 months
38. Nitrate	P, FP, G	Cool, ≤6 °C[18]	48 hours

Parameter Number/Name	Container[1]	Preservation[2, 3]	Maximum Holding Time[4]
39. Nitrate-nitrite	P, FP, G	Cool, ≤6 °C[18], H_2SO_4 to pH<2	28 days
40. Nitrite	P, FP, G	Cool, ≤6 □C[18]	48 hours
41. Oil and grease	G	Cool to ≤6 °C[18], HCl or H_2SO_4 to pH<2	28 days
42. Organic Carbon	P, FP, G	Cool to ≤6 °C[18], HCl, H_2SO_4, or H_3PO_4 to pH<2	28 days
44. Orthophosphate	P, FP, G	Cool, to ≤6 °C[18,24]	Filter within 15 minutes; Analyze within 48 hours
46. Oxygen, Dissolved Probe	G, Bottle and top	None required	Analyze within 15 minutes
47. Winkler	G, Bottle and top	Fix on site and store in dark	8 hours
48. Phenols	G	Cool, ≤6 °C[18], H_2SO_4 to pH<2	28 days
49. Phosphorous (elemental)	G	Cool, ≤6 °C[18]	48 hours
50. Phosphorous, total	P, FP, G	Cool, ≤6 °C[18], H_2SO_4 to pH<2	28 days
53. Residue, total	P, FP, G	Cool, ≤6 °C[18]	7 days
54. Residue, Filterable	P, FP, G	Cool, ≤6 °C[18]	7 days
55. Residue, Nonfilterable (TSS)	P, FP, G	Cool, ≤6 °C[18]	7 days
56. Residue, Settleable	P, FP, G	Cool, ≤6 °C[18]	48 hours
57. Residue, Volatile	P, FP, G	Cool, ≤6 °C[18]	7 days
61. Silica	P or Quartz	Cool, ≤6 °C[18]	28 days
64. Specific conductance	P, FP, G	Cool, ≤6 °C[18]	28 days
65. Sulfate	P, FP, G	Cool, ≤6 °C[18]	28 days
66. Sulfide	P, FP, G	Cool, ≤6 °C[18], add zinc acetate plus sodium hydroxide to pH>9	7 days
67. Sulfite	P, FP, G	None required	Analyze within 15 minutes
68. Surfactants	P, FP, G	Cool, ≤6 °C[18]	48 hours
69. Temperature	P, FP, G	None required	Analyze
73. Turbidity	P, FP, G	Cool, ≤6 °C[18]	48 hours
Table IC - Organic Tests[8]			
13, 18-20, 22, 24-28, 34-37, 39-43, 45-47, 56, 76, 104, 105, 108-111, 113. Purgeable Halocarbons	G, FP-lined septum	Cool, ≤6 °C[18], 0.008% $Na_2S_2O_3$[5]	14 days
6, 57, 106. Purgeable aromatic hydrocarbons	G, FP-lined septum	Cool, ≤6 °C[18], 0.008% $Na_2S_2O_3$[5], HCl to pH 2[9]	14 days[9]
3, 4. Acrolein and acrylonitrile	G, FP-lined septum	Cool, ≤6 °C[18], 0.008% $Na_2S_2O_3$, pH to 4-5[10]	14 days[10]
23, 30, 44, 49, 53, 77, 80, 81, 98, 100, 112. Phenols[11]	G, FP-lined cap	Cool, ≤6 °C[18], 0.008% $Na_2S_2O_3$	7 days until extraction,40 days after extraction
7, 38. Benzidines[11, 12]	G, FP-lined cap	Cool, ≤6 °C[18], 0.008% $Na_2S_2O_3$[5]	7 days until extraction[13]

Parameter Number/Name	Container[1]	Preservation[2, 3]	Maximum Holding Time[4]
14, 17, 48, 50-52. Phthalate esters[11]	G, FP-lined cap	Cool, ≤6 ºC[18]	7 days until extraction, 40 days after extraction
82-84. Nitrosamines[11, 14]	G, FP-lined cap	Cool, ≤6 ºC[18], store in dark, 0.008% $Na_2S_2O_3$[5]	7 days until extraction, 40 days after extraction
88-94. PCBs[11]	G, FP-lined cap	Cool, ≤6 ºC[18]	1 year until extraction, 1 year after extraction
54, 55, 75, 79. Nitroaromatics and isophorone[11]	G, FP-lined cap	Cool, ≤6 ºC[18], store in dark, 0.008% $Na_2S_2O_3$[5]	7 days until extraction, 40 days after extraction
1, 2, 5, 8-12, 32, 33, 58, 59, 74, 78, 99, 101. Polynuclear aromatic hydrocarbons[11]	G, FP-lined cap	Cool, ≤6 ºC[18], store in dark, 0.008% $Na_2S_2O_3$[5]	7 days until extraction, 40 days after extraction
15, 16, 21, 31, 87. Haloethers[11]	G, FP-lined cap	Cool, ≤6 ºC[18], 0.008% $Na_2S_2O_3$[5]	7 days until extraction, 40 days after extraction
29, 35-37, 63-65, 107. Chlorinated hydrocarbons[11]	G, FP-lined cap	Cool, ≤6 ºC[18]	7 days until extraction, 40 days after extraction
60-62, 66-72, 85, 86, 95-97, 102, 103. CDDs/CDFs[11]			
Aqueous Samples: Field and Lab Preservation	G	Cool, ≤6 ºC[18], 0.008% $Na_2S_2O_3$[5], pH<9	1 year
Solids and Mixed-Phase Samples: Field Preservation	G	Cool, ≤6 ºC[18]	7 days
Tissue Samples: Field Preservation	G	Cool, ≤6 ºC[18]	24 hours
Solids, Mixed-Phase, and Tissue Samples: Lab Preservation	G	Freeze, ≤ -10 ºC	1 year
114 -118. Alkylated phenols	G	Cool, < 6 ºC, H_2SO_4 to pH < 2	28 days until extraction, 40 days after extraction
119. Adsorbable Organic Halides (AOX)	G	Cool, < 6 ºC, 0.008% $Na_2S_2O_3$ HNO_3 to pH < 2	Hold *at least* 3 days, but not more than 6 months
120. Chlorinated Phenolics		Cool, < 6 ºC, 0.008% $Na_2S_2O_3$ H_2SO_4 to pH < 2	30 days until acetylation, 30 days after acetylation
Table ID - Pesticides Tests:			
1-70. Pesticides[11]	G, FP-lined cap	Cool, ≤6 ºC[18], pH 5-9[15]	7 days until extraction, 40 days after extraction
Table IE - Radiological Tests:			
1-5. Alpha, beta, and radium	P, FP, G	HNO_3 to pH<2	6 months
Table IH - Bacterial Tests:			
1. E. coli	PA, G	Cool, <10 ºC, 0.0008% $Na_2S_2O_3$[5]	8 hours[22]

Parameter Number/Name	Container [1]	Preservation [2, 3]	Maximum Holding Time [4]
2. Enterococci	PA, G	Cool, <10 ºC, 0.0008% $Na_2S_2O_3$ [5]	8 hours[22]
Table IH - Protozoan Tests:			
8. Cryptosporidium	LDPE; field filtration	1 - 10 ºC	96 hours [21]
9. Giardia	LDPE; field filtration	1 - 10 ºC	96 hours [21]

[1] ''P'' is for polyethylene; ''FP'' is fluoropolymer (polytetrafluoroethylene (PTFE); Teflon®), or other fluoropolymer, unless stated otherwise in this Table II; ''G'' is glass; ''PA'' is any plastic that is made of a sterilizable material (polypropylene or other autoclavable plastic); ''LDPE'' is low density polyethylene.

[2] Except where noted in this Table II and the method for the parameter, preserve each grab sample within 15 minutes of collection. For a composite sample collected with an automated sample (e.g., using a 24-hour composite sample; see 40 CFR 122.21(g)(7)(i) or 40 CFR Part 403, Appendix E), refrigerate the sample at ≤ 6 °C during collection unless specified otherwise in this Table II or in the method(s). For a composite sample to be split into separate aliquots for preservation and/or analysis, maintain the sample at ≤ 6 °C, unless specified otherwise in this Table II or in the method(s), until collection, splitting, and preservation is completed. Add the preservative to the sample container prior to sample collection when the preservative will not compromise the integrity of a grab sample, a composite sample, or aliquot split from a composite sample within 15 minutes of collection. If a composite measurement is required but a composite sample would compromise sample integrity, individual grab samples must be collected at prescribed time intervals (e.g., 4 samples over the course of a day, at 6-hour intervals). Grab samples must be analyzed separately and the concentrations averaged. Alternatively, grab samples may be collected in the field and composited in the laboratory if the compositing procedure produces results equivalent to results produced by arithmetic averaging of results of analysis of individual grab samples. For examples of laboratory compositing procedures, see EPA Method 1664 Rev. A (oil and grease) and the procedures at 40 CFR 141.34(f)(14)(iv) and (v) (volatile organics).

[3] When any sample is to be shipped by common carrier or sent via the U.S. Postal Service, 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 requirement 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_3) in 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).

[4] Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before the start of 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). For a grab sample, the holding time begins at the time of collection. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR part 403, Appendix E), the holding time begins at the time of the end of collection of the composite sample. For a set of grab samples composited in the field or laboratory, the holding time begins at the time of collection of the last grab sample in the set. Some samples may not be stable for the maximum time period given in

A Look at the
Wastewater Treatment Plant Laboratory

This handout has been prepared based on deficiencies found while performing Performance Audit inspections at wastewater treatment plants for the Tennessee Department of Environment and Conservation in the Division of Water Pollution Control. Suggestions have been made in an effort to assist the operator of a wastewater treatment plant in performing his laboratory duties.

Every wastewater treatment plant has its own distinctive characteristics and needs. Many of the observations and suggestions provided may not apply to every facility.

The following are problems associated with records and reports.

1. Failure to keep permits and records at the wastewater treatment plant.
   (NPDES Permit Part 1, 2.1.3)
   All permits and records should be kept at the wastewater treatment plant. In most cases this is a requirement in the permits.

2. Failure to keep records for three years. **(NPDES Permit Part 1, 1.2.5)**
   All records and information resulting from the monitoring activities required by the permits are to be retained for a minimum of three (3) years, or longer, if requested by the Division of Water Pollution Control. This includes all worksheets and scrap pieces of paper where calculations for analyses are performed and calibration and maintenance of instrumentation.

3. Uncertainty of what to do with records past the 3-year period.
   They can either be archived or destroyed, only if this Division has not given instructions to retain them past the 3-year period.

4. Inadequate or no worksheets on which to record data.
   Refer to permit **(NPDES Permit Part 1, 1.2.4)**

5. Illegible and unorganized data is being recorded on worksheets.
   (NPDES Permit Part 1, 2.1.3)
   All work should be written in a manner where inspectors would be able to interpret the data. Only EPA approved methods may be used to perform the required analyses.

6. Failure to document calibration and maintenance of equipment.
   (NPDES Permit Part 1, 1.2.5)
   Documentation of calibration and maintenance of equipment should also be maintained.

7. Failure to monitor parameters at the required frequencies. **(NPDES Permit Part 1, 1.2.2)**
   Parameters should be monitored at their required frequencies (according to the permit). Analyses should not be locked into specific days during the week. These days should be changed to ascertain any potential problems that may occur during an entire week.

Barbara G. Loudermilk, Chemist
TDEC, WPC, Nashville Environmental Field Office
Updated May 14, 2007

8. Unable to understand what should be recorded on Monthly Operational Reports.

   All columns on an MOR should be filled out to the best of the operator’s ability. **(NPDES Permit Part 1, 1.3.1)**

9. Uncertain whether a computer generated MOR is permissible or not.

   Should an operator want to submit his data on computer generated MORs, he should use the same format as the State provided MORs. The operator should provide both the MOR and the computer-generated MOR to the field office for approval. **(NPDES Permit Part 1, 1.3.1)**

   On the question of data storage, if it is written down on paper first then that is the permanent record and it must be maintained. Storage of data and other information generated on computer is acceptable but a very rigorous tracking system with passwords and control level f or data must be in place. Also, all records must be backed up on routine schedule and second disc or tape stored at a secure second location. These all have to be in place before electronic data is acceptable.

10. Failure to fill out DMRs correctly.

    The Discharge Monitoring Reports (DMRs) are not being filled out correctly, due to a misleading feature of the preprinted DMR form. The headings near the top of the sheet labeled (NPDES Permit Part 1, 1.3.1)

    | AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS |

    apparently have caused some misunderstanding as to the information required. The sampling intervals and units typed in the stippled gray portion to the right of each parameter listed should be followed, even where there is a conflict with the preprinted heading. For example, the entry for “Solids, Total Suspended, Effluent Gross Value”, in the fourth column to the right, the monthly average value should be entered because “MO AVG.” is typed in the stippled gray area (the preprinted column heading “MINIMUM” should be ignored.

11. Failure to calculate weekly averages correctly.

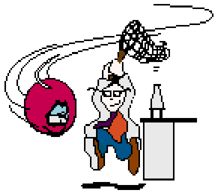
    Weekly averages should be reported by first averaging the data in each week, Sunday through Saturday, of the month then reporting the maximum value of those averages. If the week is not complete by the end of one month, then that week’s data should be reported with the month in which the week ends.

12. Failure to submit DMRs and MORs in a timely manner.

    Discharge Monitoring Reports (DMRs) and Monthly Operational Reports (MORs) frequently arrive late. DMRs should be postmarked no later than 15 days after the completion of the reporting period. (MORs) are to be submitted, in the field office, by the 15th day of the month following data collection. **(NPDES Permit Part 1, 1.3.1)**

13. Failure to have a Quality Control program. **(NPDES Permit Part 1, 1.2.3)**
    A Quality Control program should be initiated by each facility. An acceptable QC program would incorporate both quality control (precise and accurate data, e.g. duplicates) and quality assurance (how close the data agrees, statistics are commonly used). Blanks are used to indicate contamination, duplicates measure precision, and spike samples measure accuracy. Duplicates should be analyzed at a frequency of at least 1 for every 10 samples analyzed.

14. Failure to have an SOP. **(NPDES Permit Part 1, 2.1.4)**
    Each facility should also have its own Standard Operations Procedure (SOP). An SOP would provide what is typically done at the laboratory and how operations are accomplished. This would include collection of samples to the actual method used for each laboratory analyses.



THE FOLLOWING ARE PROBLEMS ASSOCIATED WITH THE LABORATORY.

1. Failure to follow approved laboratory procedures. **(NPDES Permit Part 1, 1.2.3)**
   A laboratory manual should be available for reference to EPA approved methods (e.g. **Laboratory Methods for Wastewater Analyses**, 1986, current **Standards Methods for the Examination of Water and Wastewater**). The **40 *CFR* Part 136** provides the approved test procedures for the analysis of pollutants under the **Clean Water Act.** Only approved laboratory procedures should be used. Any deviation would make self-monitoring data questionable and in some cases invalid, thus, resulting in violations.

2. Failure to properly mark containers.
   If samples from more than one wastewater treatment plant are analyzed, containers should be marked distinctly to avoid any confusion between samples.

3. Using expired chemicals in analyses.
   Chemicals should not be used beyond the expiration date. Purchase according to need. It won't be a bargain if the reagents expire before they are opened.

4. Uncertainty of when reagents have expired.
   Write down the date received or contact the manufacturer on reagents that have no expiration date.

5. Storing food and drink in ovens or refrigerators where samples are kept.
   Food and drink should not be stored in refrigerators or ovens that also contain chemicals and wastewater samples. Chemicals should be kept away from foods, as it is an unnecessary safety hazard.

6. Failure to document dates, times and initials for collection and analyses.
(NPDES Permit Part 1, 1.2.4)

The dates and times that the analyses were performed should be documented as should the name of the person who collected and analyzed the samples. This is especially important when transporting samples from one facility to another for analysis. The method of analyses should also be documented. This can either be referenced to the SOP or written directly on the daily worksheet.

7. Failure to adequately clean glassware.

Glassware should be washed thoroughly in a tub of soapy water, rinse well with tap water and follow with distilled water rinsing. Just rinsing the glassware is not considered cleaning.

8. Uncertain whether to purchase prepared reagents or prepare in-house reagents.

Some reagents such as sodium thiosulfate, 10%, used for preservation and chlorine removal for fecal coliform collection, can be prepared at the plant with minimal cost. Although prepared reagents tend to be costly they also can save valuable time.

9. Failure to properly label in-house prepared reagents.

The in-house prepared reagents should be labeled with date, time, initials, and possible health hazards. A log of the reagents prepared should be kept.

10. Failure to maintain a log for equipment. **(NPDES Permit Part 1, 2.1.4)**

A log should also be kept to record the temperatures of the ovens, waterbath and incubator. It's advantageous to place the log near the equipment being monitored, but can also be recorded on the daily worksheet. This log should include the date, time, temperature and the initials of whoever is doing the checking.

The following are problems frequently found with the <u>Settleable Solids (SS)</u> analysis.

Failure to follow EPA approved methodology.

Too frequently, deviation of the SS methodology has been found. For SS the appropriate methodology requires that the sample be shaken well, be poured into an Imhoff cone, be allowed to settle for 45 minutes, and then very slowly stirred to dislodge material adhering to the sides. The solids in the sample should then be allowed to settle for another 15 minutes then be read to the nearest 0.1 mL/L mark.

Laboratories typically have either the glass or the plastic cones. The lowest marking on the glass cone is the 0.1 mL/L mark. The plastic cones have a screw cap at the tip. The lowest marking is the 0.5 mL/L. You can't record < 0.1 mL/L if the lowest mark is 0.5 mL/L. If you happen to have a glass cone and a plastic cone, use the glass for the final and the plastic for the raw. Otherwise, you must property record what you see.

The following are problems frequently found with Dissolved Oxygen (DO) analysis.

1. Failure to run the raw DO analyses.
   Run both raw and final DO analyses and report both on the MOR.

2. Failure to collect DO sample properly.
   When collecting the sample be sure to collect the sample with a BOD bottle using the stopper. Fill the BOD bottle to near the top and then stopper immediately to avoid any further contact with the air. This assures that no air bubbles are caught between the stopper and the sample.

3. Uncertainty of which DO meter calibration to use.
   Air Calibration for the meter is acceptable by EPA. If the air calibration method is used then the membranes must be kept fresh. Change the membrane at a minimum of once per month. If air bubbles are visible under the membrane, then the membrane must be changed. Follow manufacturer instructions for proper maintenance and calibration.

   Winkler method is considered the better of the two methods used to calibrate the DO meter. Three bottles should be filled with distilled water. The first and third bottles should be titrated as required by the EPA approved method to determine the DO. The second bottle is used to adjust the setting on the meter to the averaged DO value found by titration of the other two bottles.

4. Failure to standardize the titrant.
   If the titrant, 0.0375 N sodium thiosulfate is used in the Winkler method, then it must be standardized daily according to the approved methodology.

   Another option is to titrate with a 0.03750N phenylarsine oxide (PAO) solution. This can be purchased pre-standardized. PAO solutions are stable. No further standardization would be necessary.

5. Failure to store DO probes properly.
   DO probes can be stored in a BOD bottle containing at least 1 inch of water. Refer to the operations manual for the DO probe and meter or contact the YSI service center for assistance regarding proper maintenance of the equipment. Keep this bottle clean.

The following are problems frequently found with the Biochemical Oxygen Demand/Carbonaceous Biochemical Oxygen Demand (BOD/CBOD)

1. Uncertainty of when to seed the sample.

   If an effluent composite sample is collected before disinfection, no seeding or dechlorination in the CBOD method is required.

   If an effluent composite sample is collected following disinfection, this requires seeding and, when appropriate, checking the sample for any residual chlorine prior to setting up the CBOD samples.

2. Failure to adequately dechlorinate a sample.

   Just allowing the samples to sit for a short period of time in order to dissipate any residual chlorine is not as effective as chemical de-chlorination. Chemical dechlorination also eliminates the chlorine in a shorter period of time. Failure to properly dechlorinate would result in artificially low results.

3. Failure to allow the samples to come to room temperature before making dilutions.

   Since composite samples are kept at 6^{o}C or below, the samples should be warmed to 20 ± 1^{o}C (comparable to 68^{o}F) before making dilutions. This may be accomplished by allowing the samples to come to room temperature (approximately 68^{o}F) or by setting the sample bottles in a warm water bath. Samples with initial DO's greater than 9.0 mg/l at $20\ ^{o}$ C (e.g. stream samples) are considered supersaturated with oxygen. These samples may be vigorously shaken or aerated with clean, compressed air to bring down to saturation, less than or equal to 9.0 mg/l. Refer to Standard Methods for additional information. The samples should be brought to room temperature, set up and analyzed within two hours of collection.

4. Introducing contamination into the sample.

   Cross-contamination, which can produce inaccurately high results, can be avoided by properly rinsing the graduated cylinders between measuring sample volumes. Specific graduated cylinders can be delegated for each influent, effluent and stream sample. Clean, Clean, Clean!!!

5. Failure to adequately stir the sample prior to making dilutions.

   Sample should be stirred thoroughly to obtain a representative sample.

6. Failure to set up the appropriate amount of dilutions.

   At least three dilutions should be set up for both influent and effluent samples with a duplicate performed on every tenth sample.

7. Uncertainty of the purpose of the glucose-glutamic acid check.

    To check the quality of the dilution water it is recommended that a glucose-glutamic acid check be implemented. A typical value of 198 PPM ± 30.5 has been found for the glucose-glutamic acid concentration. This should be done periodically along with a known sample, if possible, in order to establish laboratory control limits.

    A low value could be indicative of a toxic affect caused from trace metals in the dilution water (e.g. copper).

    A high value could be indicative of several factors (e.g. contamination from improper rinsing of glassware after cleansing and imprecise measurement of standards).

    Should the measurement fall outside the previously given range, the tests should be reported and flagged as to the findings. The problem should then be investigated in order to determine and eliminate the source.

8. Failure to follow the BOD criteria for reporting results.

    Follow the BOD criteria for reporting the results. EPA approved methodology states that the initial DO must be less than or equal to 9.0 mg/l. The samples must deplete at least 2.0 mg/L DO and must leave 1.0 mg/L final DO. Results from dilutions that do not meet these criteria are considered invalid and should be discarded.

    The blank oxygen depletion should be 0.2 mg/L or less as required by EPA approved methodology. Otherwise, an investigation should be initiated to determine the cause. Check for soap residue due to improper rinsing. The results still can be reported but must be marked that the blank is outside the guidelines.

9. Uncertainty of which nitrification inhibitor to use.

    There are two nitrification inhibitors available for use in the CBOD analysis method, **2-Chloro-6 (Trichloromethyl) Pyridine** and **Nitrification Inhibitor, Formula 2533^{TM}**. The biggest difference between the two is that Formula 2533^{TM} is more soluble than the other, thereby yielding better results.

    The dispenser cap for the nitrification inhibitor is well worth the money.

10. Failure to properly monitor the temperature of composite samplers.

    The temperature of the composite samplers are not generally monitored or maintained at the required temperature of 6°C. During the collection of composite samples, the temperature of the samplers must maintain temperatures at 6°C or below (but above freezing 0°C).

11. Failure to properly monitor the temperature of the BOD incubator.
    The BOD incubator temperature must be maintained at 20° ± 1°C. A log should be kept for each piece of equipment to record the date, time, temperature, and the initials of whoever was checking it. It is just as easy to record the temperature on the daily worksheet.

The following are problems frequently found with the pH analysis.

1. Failure to compensate for temperature.
   Since pH is temperature dependent, an automatic temperature compensator (ATC) probe must be use or the temperature measured and manually set on the instrument.

2. Failure to properly store the pH electrode.
   Store electrodes according to manufacturer instructions! Orion states using pH 7 buffer with 1 gram of KCl (potassium chloride).

3. Using expired pH standards.
   Do not use expired pH standards. Plan to buy what can be used before the expiration date.

4. Failure to properly calibrate the meter.
   A two-point calibration is a requirement. A two-point calibration brackets the normal pH range found at the plant. It provides a line on which the best possible data point for the sample can be found. If the sample falls outside the calibrated range then the instrument should be recalibrated.

   If you have the capability to perform a 3-point calibration, follow manufacturer procedures.

5. Failure to use fresh standard solutions for calibration of the pH meter.
   Prepare fresh standard solutions for each daily use for calibration. If stock bottles are used, be sure to cap the bottle after pouring out an amount used for standardizing. **Never** pour used reagent back into the stock bottle. This is a big source of contamination. This method could jeopardize the integrity of the sample, which is directly related to the calibration of the meter.

6. Improper calibration procedure used in calibrating the pH meter.
   Start by adjusting the standardize (calibrate) knob with a pH 7 buffer. The slope or calibrate function on the meter should be set with a second buffer. This buffer depends on the desired range, either above pH 7 (e.g. pH 10) for the higher pH range, or below pH 7 (e.g. pH 4) for the lower pH range.

7. Failure to rinse the electrode prior to reading the sample.
   Be sure to rinse the electrode well after standardizing the meter and prior to reading the pH of the sample. This eliminates cross-contamination.

8. Failure to stir the sample.
   Stirring the sample at a rate of about one revolution/second is necessary in obtaining a quick and accurate reading.

9. Failure to run the sample immediately after collection.
   Don't collect the pH sample until it is ready to be run. The analysis should be run immediately. According to EPA, this generally means within 15 minutes of collection.

10. Failure to have a backup probe.
    An extra pH probe should be available in the event that the current probe malfunctions.

11. Uncertainty of how to maintain an electrode.
    The electrolyte should be added to the new electrode, through a filler hole in the side, when it is ready for use. After the electrolyte has been added, it should not be used for at least one hour. Be sure to rinse off any excess electrolyte and pat dry the electrode. Do not rub the electrode vigorously because it could affect the probe. This will assure that none of the electrolyte is introduced into the sample. The electrolyte is a caustic that causes an obvious increase in the pH.

    Be sure to take the filler hole cap off when taking readings. This cap may be replaced when not in use to prevent evaporation of the electrolyte.

The following are problems frequently found with the <u>Total Suspended Solids (TSS)</u> analysis.

1. Failure to adequately shake the sample.
   The biggest problem with the TSS is that operators are not shaking the sample well enough prior to analysis. This is necessary to obtain a representative sample.

2. Uncertainty of why results are too **high**. (When comparing results to State results.)
   Frequently, sample volumes are too high for the amount of solids in the sample. This requires longer filtration periods for elimination of the liquid. Prolonged filtration times may produce high results due to excessive solids on the clogged filter. By reducing the sample size, more satisfactory results should be obtained.

   A constant weight must be demonstrated prior to reporting the results. This is to ensure that all the moisture has been eliminated. If this is not accomplished then erroneously high results may be obtained.

   The drying oven should be kept clean to minimize contamination of samples.

3. Uncertainty of why results are too **low**. (When comparing results to State results.)

   Solids can be lost under the filter paper when pouring in the sample. Wetting the filter paper with distilled water prior to pouring the sample will lessen the possibility of the solids being lost under the filter rather than collected on the filter.

   Filtered samples should not be placed directly on the oven rack. The filters could stick to the rack and lose fibers, which could change the weight of the sample. They could also gain contaminants or spill the filter contents. Aluminum weighing dishes are recommended.

4. Lack of good laboratory technique.

   Don't use fingers to pick up crucible or filter. The oil on your fingers adds weight. Use tongs instead.

5. Uncertainty of when to change the desiccant.

   Make sure that the color indicator desiccant is changed when the color turns to pink.

   An option would be to obtain the non-color indicator and mix it with the color indicator desiccant. This would incorporate cost with performance.

6. Failure to properly monitor the temperature of the drying oven.

   The oven should be maintained at a temperature between 103 - 105^{o}C at all times.

7. Failure to perform quality control procedures

   A duplicate should be performed once every 10th sample.

   A distilled water blank should be analyzed with every 10th sample for quality assurance.

The following are problems frequently found with the <u>Total Chlorine Residual</u> analysis.

1. Failure to calibrate the instrument prior to use.

   The Instrument should be calibrated prior to each use. Chlorine or $KMnO_4$ standards may be used. There are several companies that manufacture pre-made standards.

2. Uncertainty of the proper method to use.

   The permit limit must be considered when deciding the appropriate method to use. The low limits presently being given require the lowest possible detection limits that a method can provide. DPD, electrode and amperometric methods are currently the methods that provide the lowest detection limits available.

   Iron, manganese, nitrates and organic mercaptans can interfere with DPD readings giving a false positive for chlorine. Some facilities are unable to use the colorimeter due to these interferences.

   The amperometric method requires greater skill than the colorimetric method but there are less problems with these interferences.

3. Uncertainty of what the lowest reportable value should be.

The amperometric method requires greater skill than the colorimetric method but there are fewer problems with these interferences.

The current instrument detection limit is 0.05 mg/L. Many permit limits lie below this value. Many operators have been reporting <0.1 mg/L. This was the old detection limit and should not be used anymore. If the value in the permit limit is within the capability of the instrument, it is preferable that the operators report the actual value that is obtained.

Be aware that the detection limit can change and become even lower as the technology improved instruments become available.

The following are problems frequently found with the Ammonia as Nitrogen (NH_3-N) analysis.

1. Failure to properly preserve samples.

Samples should be preserved properly with acid when the analysis is not performed immediately; this generally means within 15 minutes. (1.0 mL of sulfuric acid per liter of sample.)

2. Failure to adequately shake the sample.

Thoroughly shaking the sample is necessary to obtain a more representative sampling.

3. Failure to distill samples.

Distillation is required to remove impurities that would interfere with the analyses and produce erroneous results. Contrary to popular belief, the probe method does not eliminate the need to distill (as stated in Standard Methods). The 40CFR part 136 takes precedence over the Standard Methods. It requires distillation for all methods unless comparability has been shown.

Also, as footnote 6 states for Table 1B, 40 CFR part 136, “Manual Distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary; however, manual distillation will be required to resolve any controversies.”

Remember, this is a statistical analysis. The guidance that we provide says to collect 3 samples over a given month. Then, each sample be divided into replicates to be distilled and undistilled before analysis.

To do this, we recommend that you preserve the sample that you collect for the “undistilled” analysis. By the way, this allows you 28 days in which to analyze the sample. Good to remember if you every have a problem.

Oh, and if you decide to distill, it’s suggested that the distillation apparatus be set up in a place where it can remain (preferably under a working ventilation hood) and doesn't have to be dismantled after every use. This will eliminate a few headaches.

Did you say Ammonia-free water? Hopefully, someone has found a something out there to assist in this matter. Personally, I've had difficulty coming up with something. So, next best consideration. Let's limit the exposure to the atmosphere. Remember, not only does the air that we breathe have oxygen,, it also has nitrogen which is a component of ammonia. So, when you distill the water minimize the exposure to the air as it is distilled off.

4. Uncertainty of when the distillation procedure may be omitted.
   If distillation is to be omitted, comparability study must be performed and the data kept on file at the facility. The undistilled sample results must lie within ± 10 % of the distilled sample results. If so then the distillation process may be omitted. The comparability data should be repeated periodically (minimum once per year).

5. Failure to properly calibrate the instrument.
   Instrument calibration is required prior to running the analysis. At least two standards should be used to bracket the sample. If the sample falls outside this range, then the sample can be diluted or new standards prepared.

The following are problems frequently found with Fecal Coliform analysis.

1. Failure to use the approved method.
   Only use approved methods outlined in the 40CFR part 136. Don't get confused with total Coliform.

2. Failure to use a microscope with the appropriate magnification.
   A microscope with magnification of 10X to 15X is required to obtain a valid colony count. This magnification is necessary to determine the presence of small colonies in a sample that might otherwise be missed.

3. Failure to monitor the temperature of the water bath.
   Maintain the temperature of the water bath at a uniform and constant temperature of 44.5 ± 0.2 ^{o}C at all times.

   The thermometer must be graduated in 1/10 of ^{o}C which is needed for the ± 0.2 ^{o}C and **kept** clean.

4. Failure to follow proper sterilization procedures.
   0.1 mL of a 10% sodium thiosulfate solution should be added to each sample bottle prior to sterilization. Do not use the preservative N-10 (0.1005-0.0995) sodium thiosulfate. This is potentially harmful to the fecal coliform culture since this is stronger than the 10% (approximately 0.03N) solution stated in the EPA approved procedures.

   The sterilization procedure should be done for 15 - 20 minutes at 121^{o}C (250^{o}F).

If you have to use a pressure cooker as an autoclave, do not close the petcock until steam comes out of the ports. When the sterilization period is complete, turn steam supply off; allow glassware to slowly cool before removing.

5. Failure to properly collect the sample.

   While collecting the sample in the sterilized bottle, care must be taken to eliminate other sources of contamination (e.g. unsterilized dippers used to pour the sample into the sterilized sample bottles).

6. Uncertainty whether to use distilled water in place of buffered dilution water.

   Do **NOT** use distilled water in place of the buffered dilution water. EPA approved methodology requires the use of buffered dilution water in the procedures. The method shows you how to prepare this.

7. Failure to prepare the appropriate number of dilutions.

   Many operators are preparing only one dilution. Prepare three dilutions of the sample in order to obtain a 20 to 60-colony count.

8. Performing analysis with faulty equipment.

   Avoid using a leaking filter apparatus. This could give erroneously low results.

9. Failure to perform quality control

   It is recommended that a positive control sample be set up at least once per month as a quality control check (e.g. 1 mL of effluent prior to chlorination).

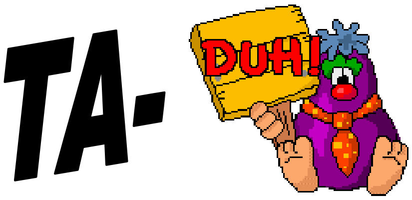
   Perform duplicate samples at least once every 10 samples.

10. Failure to perform analyses due to high flow conditions.

    Sampling during high flow events occasionally has been avoided by the operator due to frequently obtaining TNTC (Too Numerous To Count) data. (This is a violation of the permit since representative sampling is required at a specified frequency.) At several plants this occurrence is the norm rather than the exception. Operators who have worked at these plants for a number of years can probably guess as to the appropriate dilutions that could be used to obtain 20 - 60 colony counts. It's better to indicate what actually happens at the plants rather than indicate no problems. These problems would lend proof that there is a need for plant renovations.

11. Failure to report data correctly.

    There are specific guidelines that should be followed when reporting data. TNTC is not to be reported on the MOR. These guidelines are provided upon request or may be found in ***EPA Microbiological Methods for Monitoring the Environment Water and Wastes,*** EPA-600/8-78-017, December 1978.



The following are problems frequently found with the E. coli analysis.

1. Failure to use the methods approved for wastewater.
   Actually, TA-DA!

   The new Federal Register just came out in March 2007. You have the Colilert, Colilert 18 and mColiBlue 24 approved for E. coli analyses. Yipee! Well, there are others listed as well. But, these particular methods have fought long and hard for “approval”.

2. Failure to use appropriate temperature.
   Follow instructions. Don’t assume E. coli can be used at the same temperature as fecal coliform or visa versa.

Section 5
pH

pH

pH

- One of the most important and frequently used tests in water chemistry
- A measure of the intensity of the acidic or alkaline character of a solution
- Logarithmic scale of ionic activity
  0 to 14 s.u.
- pH values cannot be averaged

pH Measurement

- pH is typically measured with a meter and probe
- This is an electrochemical method of analysis

pH Theory

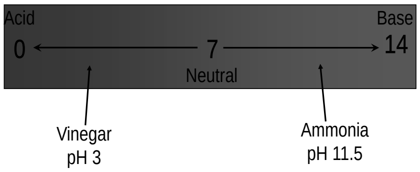
- Acid
  - increases the hydrogen ion (H+) concentration in a solution
- Base
  - increases the hydroxide ion (OH-) concentration in a solution

pH Theory

- pH is defined as the negative log of the molar hydrogen ion concentration in aqueous solution

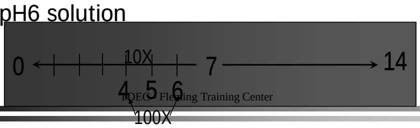
$$\mathrm{pH} = -\log[\mathrm{H}+]$$

pH Scale



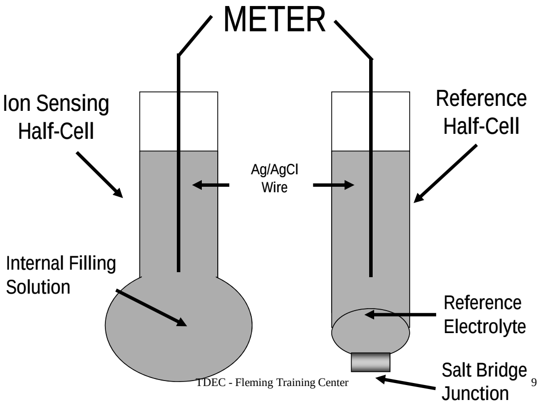
pH Scale

- pH is a negative logarithmic function
- Each decrease in pH unit = 10X increase in acidity
  - Solution at pH4 is 10X more acidic than solution at pH5
  - Solution at pH 4 is 100X more acidic than pH6 solution



How Does a pH Probe Work?

- Probe measures hydrogen ion concentration
  - Two electrodes in probe:
    - a) sensing half-cell,
    - b) reference half-cell



METER

Ion Sensing Half-Cell

Reference Half-Cell

Ag/AgCl Wire

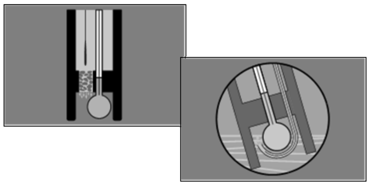
Internal Filling Solution

Reference Electrolyte

Salt Bridge Junction

Reference Half-Cell

- Dispenses reference solution which completes circuit for meter

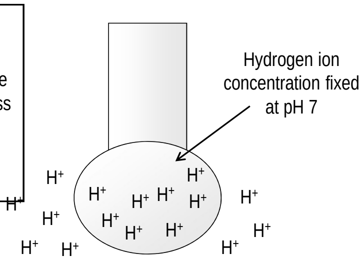


Sensing Half-Cell

pH 7 Solution

H^+ conc. the same both inside and outside glass bulb

*No potential develops



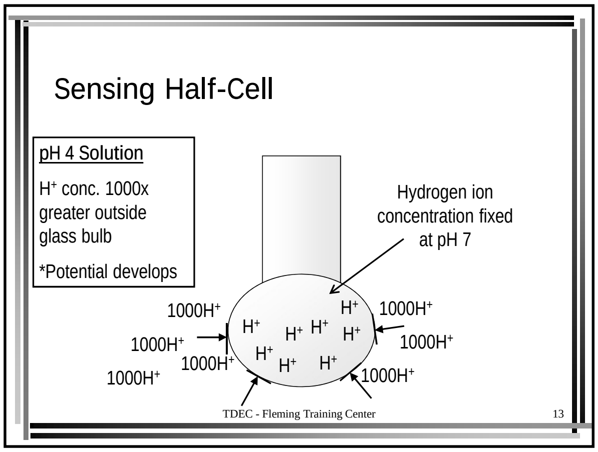
Hydrogen ion concentration fixed at pH 7

Sensing Half-Cell

pH 7 Solution

H^+ conc. the same both inside and outside glass bulb

*No potential develops

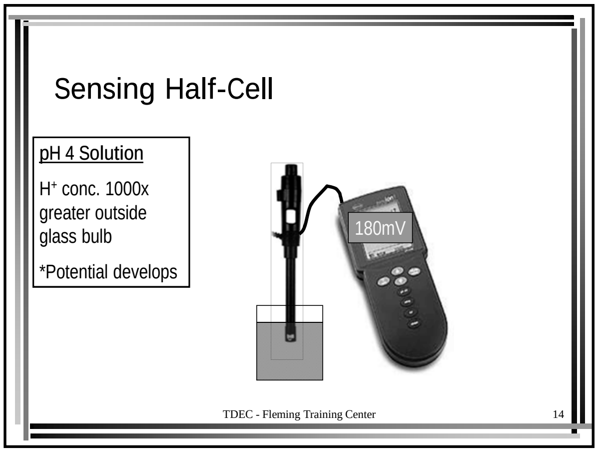


Sensing Half-Cell

pH 4 Solution

H^+ conc. 1000x greater outside glass bulb

*Potential develops

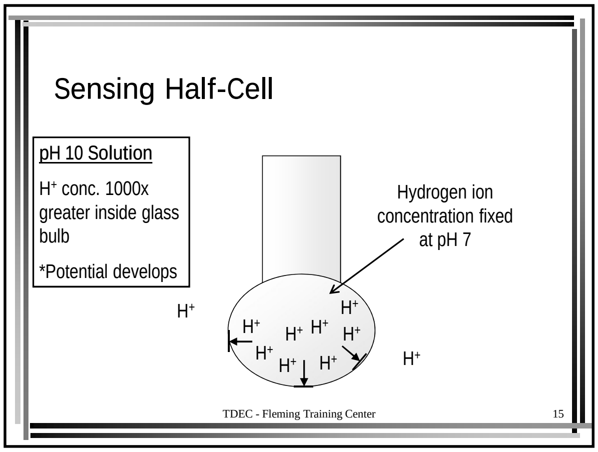


Sensing Half-Cell

pH 4 Solution

H^+ conc. 1000x greater outside glass bulb

*Potential develops

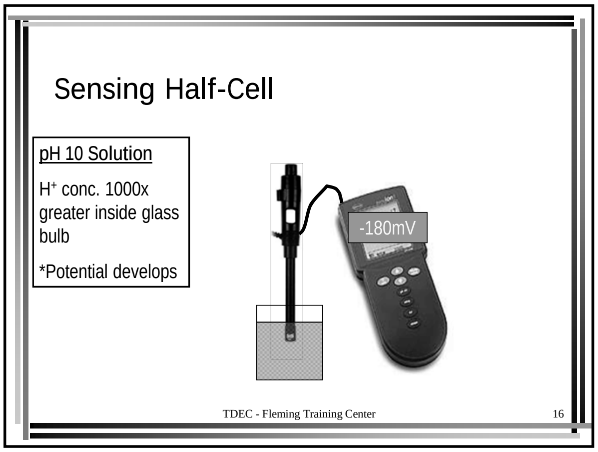


Sensing Half-Cell

pH 10 Solution

H^+ conc. 1000x greater inside glass bulb

*Potential develops

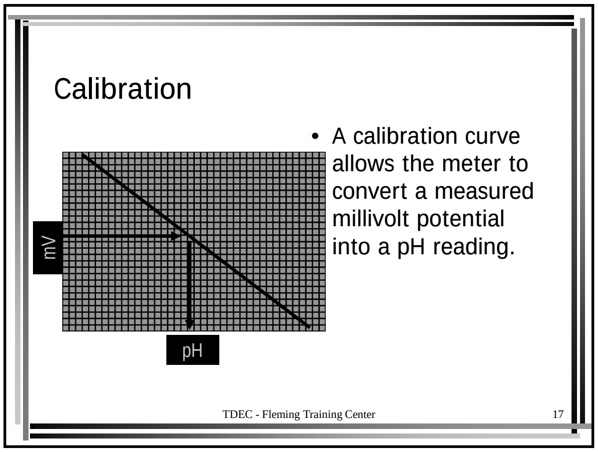


Sensing Half-Cell

pH 10 Solution

H^+ conc. 1000x greater inside glass bulb

*Potential develops



Calibration

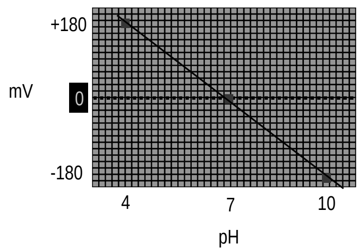
- A calibration curve allows the meter to convert a measured millivolt potential into a pH reading.

Calibration

- The optimal slope for pH is
  -58±3 mV/decade.

What does this mean?

Calibration



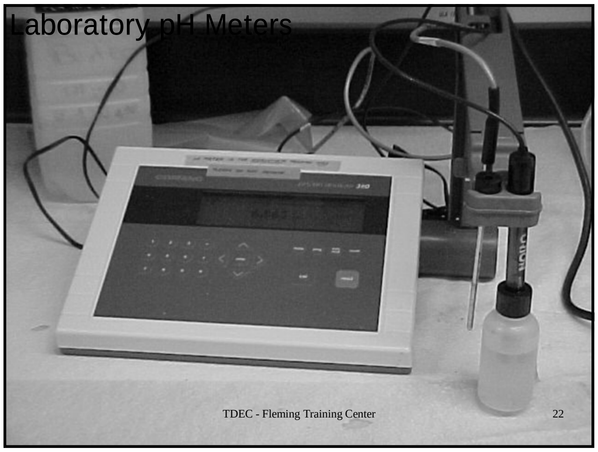
Calibration

- -180mV difference measured between pH4 and pH7
- pH4 to pH7 (3 pH units) is 1000x concentration change
- Decade = 10-fold concentration change
  = 1pH unit
- -180/3 = -60 ≈ -58mV/decade

Importance of pH control

- Ammonia toxicity is influenced by pH
- pH plays an important role in the solubility of metal salts
- pH affects the rate at which chlorine reacts to form chloramines (which are less effective disinfectants)

Laboratory pH Meters



pH Sampling

- Holding time = 15 minutes
- Preservation = none
- Sample container = glass or plastic
- Grab sample
- Continuous monitoring possible

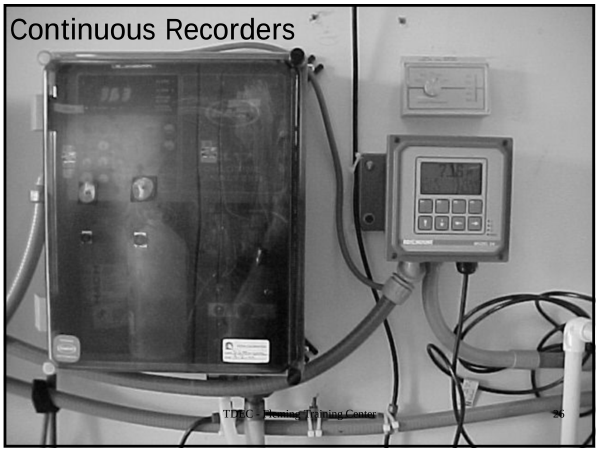
pH Meter Calibration

- Follow manufacturers instructions
- Use fresh buffers (4, 7, & 10 s.u.)
- Stir buffers and samples at the same speed without a vortex
- Rinse and blot dry electrodes between samples and buffers
- Accurate and reproducible to within 0.1 s.u.

pH Meter Calibration (cont.)

- Start with pH = 7.0 buffer (usually)
- Second buffer 3 s.u. different that brackets expected sample pH (4 or 10)
- Immerse in a third buffer - reading should be within 0.1 s.u.
- If response is accurate - read and record previous buffers as samples (pH and temperature)

Continuous Recorders



Continuous pH Monitoring EPA Method 150.2

- Immersion type electrodes easily removed from mounting :
  - Should be calibrated with two buffers that bracket expected pH and are at least 3 pH units apart
  - Adjustments made until readings are within ±0.1 s.u. of buffer value
  - Calibration must be made at least daily

Continuous pH Monitoring EPA Method 150.2

- Immersion type electrodes not easily removed from mounting :
  - Indirect procedure - System must agree within 0.1 s.u. of a calibrated lab meter on an effluent sample
  - Indirect calibration once per day
  - Recalibration - System should be calibrated against two fresh buffers at least every 30 days and more often if needed

COMMON DEFICIENCIES

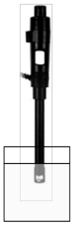
- The pH meter was calibrated using one buffer or expired buffers
- The continuous pH meter was not calibrated on a regular basis
- Buffers were left open and being reused for a week

Maintenance

- New probe
- Calibration
- Measurement/Storage
- Troubleshooting
- Cleaning

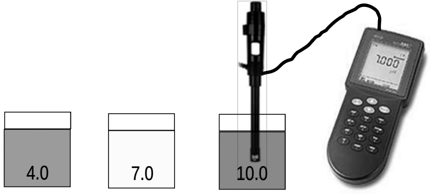
New Probe

- Condition new pH probe in pH 7 buffer for approximately 30 minutes before initial use
- Probes must also be conditioned after cleaning or if it had been stored dry as long term storage



Calibrate

- Calibrate pH meters daily using two or three buffer solutions



Measurement

- Place probe into sample, stir, and wait for readings to stabilize
- Rinse and dry between measurements
- Storage between measurements
  - Sample *or* solution of similar ionic strength to sample
  - pH7 buffer
  - pH electrode storage solution *(best)*

Measurement - Special Considerations

- Low ionic strength solutions
  - Very clean samples with low TDS
  - Absorption of atmospheric carbon dioxide is a major source of drift
    - Low ionic strength sample chamber
    - Condition electrode in a solution of comparable ionic strength before use

Troubleshooting

- mV reading in pH 7 buffer
  - Should read 0 ± 30 mV in pH 7 buffer
- Response time
  - May require cleaning if slow in buffered solution
- Slope
  - Optimal slope is -58 ± 3 mV/decade

Cleaning

- Slow response may indicate need for cleaning
  - Alternate soaking in dilute hydrochloric acid and dilute sodium hydroxide
  - Rinse with deionized water
  - Condition in pH 7 buffer before use

- Read probe manual for cleaning method recommended by manufacturer

Fleming Training Center

pH Calibration Record

Date	Time	Temperature of Buffers	Slope	Buffers Used			Technician	Remarks
				4	7	10		

Month____________

pH Meter ________________

Fleming Training Center

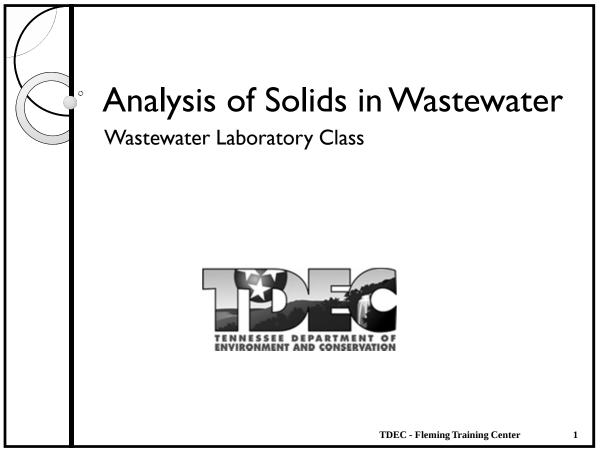
pH Sample Record

Date	Time	Location	Temp. of Solution	Date of Last Calibration	Measured pH	Technician

Month____________

pH Meter ________________

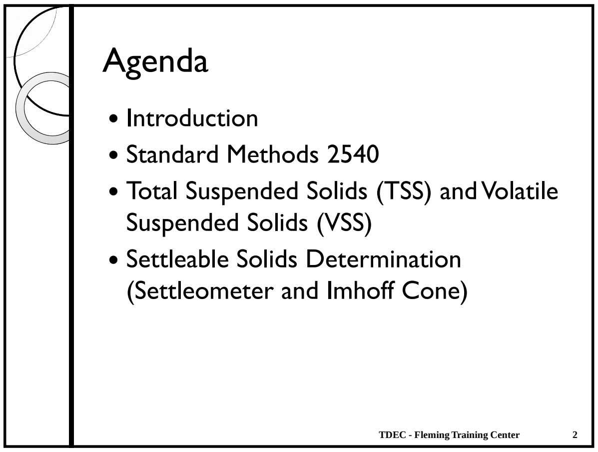
Section 6
Solids



Analysis of Solids in Wastewater

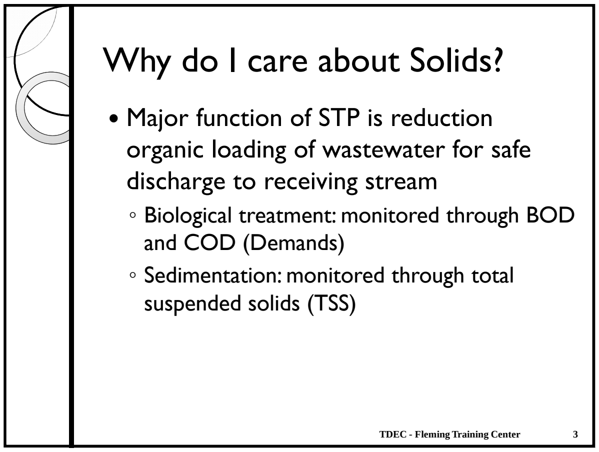
Wastewater Laboratory Class

TENNESSEE DEPARTMENT OF ENVIRONMENT AND CONSERVATION



Agenda

- Introduction
- Standard Methods 2540
- Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)
- Settleable Solids Determination (Settleometer and Imhoff Cone)

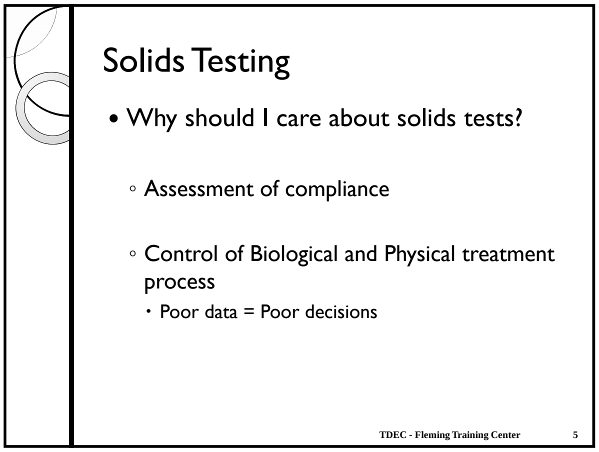


Why do I care about Solids?

- Major function of STP is reduction organic loading of wastewater for safe discharge to receiving stream
  - ◦ Biological treatment: monitored through BOD and COD (Demands)
  - ◦ Sedimentation: monitored through total suspended solids (TSS)

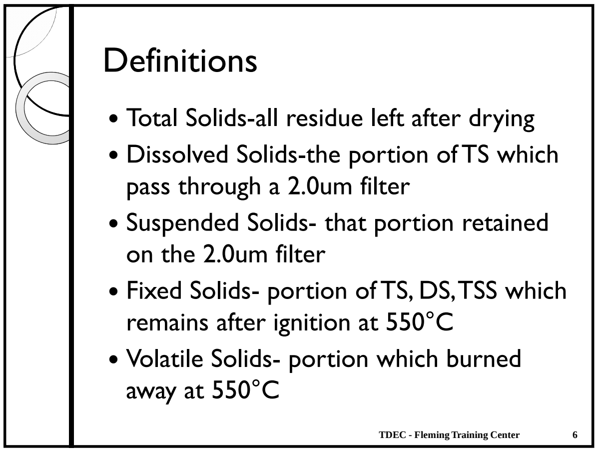
Wastewater Solids Removal

- Most suspended solids are organic
- Oxygen Demand
- Serve as refuge for harmful bacteria
- Unsightly appearance



Solids Testing

- Why should I care about solids tests?
  - ◦ Assessment of compliance
  - ◦ Control of Biological and Physical treatment process
    - Poor data = Poor decisions



Definitions

- Total Solids-all residue left after drying
- Dissolved Solids-the portion of TS which pass through a 2.0um filter
- Suspended Solids- that portion retained on the 2.0um filter
- Fixed Solids- portion of TS, DS, TSS which remains after ignition at 550°C
- Volatile Solids- portion which burned away at 550°C

Standard Methods 2540B-G

- Sources of error
  - Sampling
  - Sub sampling
  - Measuring
  - Filter/Bowl preparation
  - Filter/Bowl handling
- Remedies
  - MIX WELL
  - Measure quickly

Standard Methods 2540B-G

- Temperatures
  - Each Method has a specified drying Temp.
  - Minimize opening desiccator
  - Weigh quickly, dry samples attract moisture
- Rinse Water
  - Type III, distilled or deionized water

Standard Methods 2540B-G

- Fats, Oil, and Grease
  - May interfere due to difficulty in drying to a constant weight in time range.
- Duplicates should weigh within 5%
- Weigh to a constant weight, within 4% or 0.0005g, whichever is less.

Standard Methods 2540B-G

- Sampling
  - Glass or plastic containers, watch for particles adhering to container walls especially plastic.
- Begin test ASAP is preferred.
- Sampling Holding
  - Preserve at 4°C to minimize bio. decomp.
  - Hold <24 hours preferred
  - Never hold over 7 days

Method Choice

- Methods B-F
  - Potable, surface, saline, domestic and industrial wastewater up to 20,000mg/L
- Method G
  - Solid and Semisolid samples >2%
  - Biosolids, per 40 CFR 503

Total Solids 2540-B

- Temperature 103-105°C
- Calculations based on sample volume

Dissolved Solids 2540-C

- Temperature 180°C
- Non-regulatory at 103-105°C

Total Suspended Solids 2540-D

- Temperature 103-105°C
- Interferences
  - Limit sample size to 200mg of residue, small filter
  - Avoid prolonged filtration times >5-7 min.
- Glass fiber filter 22-125mm diameter.
  - Whatman 934AH 1.5um
  - Gelman A/E 1.0um
  - Millipore AP40
  - E-D Scientific Grade 161

Total Suspended Solids 2540-D

TSS 2540-D

- Filter preparation:
  - Pre wash approved glass fiber filter by rinsing three times with 20mL of deionized (DI) water
  - Dry in weighing pans at 103 – 105° C for 1 hour and cool in desiccator
  - Record the initial weight of the filter & pan after drying
  - Repeat this cycle of drying, desiccating and weighing until a constant weight has been reached

TSS 2540-D

- Sample Analysis:
  - Choose a sample volume to yield between 2.5 - 200 mg dried residue
  - Assemble filtering apparatus and filter
  - Wet filter with small amount of distilled water to seal before applying the sample
  - Sample must be well mixed before applying to filter

TSS 2540-D

- Sample Analysis – continued:
  - Wash filter and apparatus three times with 10 mL of DI water
  - Transfer filter into support pans
  - Dry in oven at 103-105 ° C for 1 hr and cool in desiccator
  - Weigh filter until a constant weight is obtained

TSS 2540-D

- Uses
  - Influent and effluent regulatory tests
  - Mixed Liquor Suspended Solids MLSS
  - Return Activated Sludge RAS
  - Clarifier Suspended Solids
  - Stream samples
  - Some digester solids tests

TSS 2540-D

Sample	Common Range, mg/L
Influent	150 – 400
Primary Effluent	60 – 150
Secondary Effluent	10 – 60
Tertiary Effluent	0.0 – 3.0
MLSS	1,000 – 5,000
RAS	2,000 – 12,000

TSS Calculations

- TSS in mg/L is equal to the amount of residue retained on the filter in mg per liter of sample

- Formula: $\text{TSS mg/L} = \dfrac{(A - B) \times 1{,}000{,}000}{\text{sample volume mL}}$

- Where: A = wt. of filter + dried residue (g)
  B = initial wt. of filter (g)
  1,000,000 = conversion factor
  (1 g = 1000 mg & 1L = 1000 mL)

TSS Calculations

- Wt. of filter + residue = A = 1.0215 g
- Wt. of filter = B = 1.0160 g
- Sample volume = 200
- Mg/L = 1.0215 – 1.0160

TSS – Portable and Inline

Settleable Solids 2540-F

- Important process control test for rates and volume of sedimentation
- Report as mL/L
  - Minimum Detection Limit 0.1mL/L
  - Adjust for water layers if present
- Basin control: Imhoff cone
  - Sample volume: 1 L
  - Solids settle 45 min
  - Gently stir sides
  - Solids settle another 15 minutes
  - Read after a total of 60 min

Settleable Solids 2540-F

Sample	Common Range, mL/L
Influent	8 – 20
Primary Effluent	0.1 – 3
Secondary Effluent	0.1 – 0.5

Total Solids 2540-G

- For solid and semisolid materials
  - Required or Biosolids per 40 CFR 503
- Calculations based of wet and dry weight.

Fixed and Volatile 2540-E

- An additional step of the Total, Suspended, or Dissolved Solids test.
- Sample is ignited at 550°C for 1 hr.
- Fixed Solids or Ash remains
- Volatile Solids were burned away.

Balance Operation

- Key to quality solids tests
- Care of Balance
  - Vibration, heat, sunlight
  - Dust, dirt, moisture
- Calibration
  - Outside contractor
  - In house, "S" class weights

TSS Sample Worksheet

Sample	Dish Number	Filter Weight, g (B)	Sample Volume, mL (C)	Filter + dried sample, g (A)	Filter + dried sample, g (2nd)	TSS = A-B/C
Blank			100			
Effluent			100			
Raw			25			
Mixed Liquor			5			
Dup Raw			25			
Blank			100			

Section 7
WET Testing

Whole Effluent Toxicity

WET Testing

TENNESSEE DEPARTMENT OF ENVIRONMENT AND CONSERVATION

Approaches to Evaluating Environmental Samples

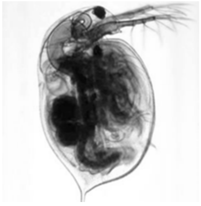
- Test sample for specific chemical substances dangerous to environment
  - Laboratory knows what to look for in sample
  - Identification techniques specific to analytes
- May overlook a compound harmful to environment

Approaches to Evaluating Environmental Samples

- Treat a living organism with a portion of sample and see if it exhibits effects:
  - Many samples screened quickly
  - Results somewhat dependent on species used in tests
  - If toxicity found, no indication of exact cause of toxicity
  - WET Testing

Test Organisms and Methods

- Methods for compliance with NPDES permits in 40 CFR 136.3
  - *Ceriodaphnia dubia* (invertebrate microcrustacean)
  - *Pimephales promelas* (vertebrate fish)



WET Test Organisms

- Lab verifies cultures are disease-free and maintain certain level of toxicant sensitivity
  - Water flea: organisms no more than 24-hrs old and all born within 8 hrs
  - Minnow: larvae < 48-hrs old and all born within 24-hr time period

Sampling

- Collected as composite over 24 hrs
- Chilled to 4°C during and after sampling
- Hold time 36 hrs from last aliquot collection until beginning of testing
- Sample size: 4L or 1gal "cubitainer"

Sample Preparation

- Effluent alkalinity, hardness, conductivity, residual chlorine, pH, etc. determined
- Chronic tests conducted by filtering sample through 60-μm plankton net
- Example: Undiluted sample (100%), 50%, 25%, 12.5%, and 6.25 (or less for no-effect concentration) are tested

Water Flea Tests

- Test chamber: 30-50 mL plastic cups, each with 15 mL and 1 organism
- Minimum 10 replicates for each concentration for total of 50 test organisms and 10 controls
- Reference toxicant often run concurrently (sodium chloride) for quality control
- Food added to each chamber
- **Incubate at 25°C; 16 hrs light/8 hrs dark**

Water Flea Tests

- Next day, surviving creatures and offspring are counted and recorded
- Living test organisms transferred to clean test chamber
- Food is added and organisms are incubated
- Test continues 6-8 days
- Total number surviving organisms for each test are tallied with number of broods and total offspring for each adult

Minnow Testing

- Test chambers are 500-1000mL glass beakers containing 250mL test solution
- Each beaker contains 10-15 embryo fish
- Usually 4 replicates for each concentration
- Reference toxicant (copper sulfate) often run for quality control
- Food added to each chamber
- **Incubate at 25°C; 16 hrs light/8 hrs dark**

Minnow Testing

- Next day, dead fish are removed
- Test chamber cleaned of debris with siphon
- Fresh test solution added to bring level back to original volume
- Food is added; test chamber put in incubator

Minnow Testing

- Test continues for 7 days
- Surviving fish for each replicate tallied
- Growth determined by placing all surviving fish for each replicate in tared weigh boat, drying at 60°C for 24 hrs or 100 °C for 6 hrs
- Average growth reported to nearest 0.001 mg

Quality Assurance

- Control organisms: survival ≥ 80%
- *Ceriodaphnia*
  - At least 60% of control adults must have produced three broods by 7±1 days
  - Surviving control adults must produce at least 15 offspring
- Fathead minnows
  - Average dry weight of controls at 7 days must be 0.25 mg per fish or greater
  - If minimum control requirements not met, tests are invalid and must be repeated

Interpretation of Results

- Data processed and plotted to determine:
  - NOEC- maximum concentration of effluent that gives no statistical difference from controls
  - LOEC- lowest concentration that produces a statistically significant difference between controls and test samples
  - IC25/50- toxicant concentration that produces 25 or 50% reduction in effect

Acute Bioassay

- Conducted with *Pimephelas promelas* and *Ceriodaphnia dubia*
- Determine "end of pipe" conditions
- Effects in 100% effluent
- Last 48-96 hrs
- Objective: determine effluent concentration that causes 50% lethality during short term exposure

Interpretation of Results

- If result of a WET test on the effluent is at or near the permit limit , you have a violation
- Retest conducted
- If toxic effect consistently found, a Toxicity Reduction Evaluation (TRE) is conducted

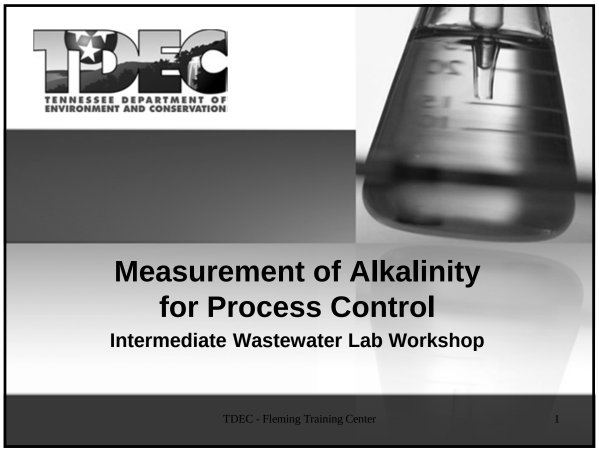
Toxicity Reduction Evaluation (TRE)

- Systematic evaluation of effluent
- Determine sources of toxicity and how to control toxicity
- May include chemical screening, process reviews, evaluation of plant performance, and toxicity identification evaluation (TIE)

Toxicity Identification Evaluation (TIE)

- Effluent samples are manipulated to remove suspect chemicals
- Samples re-tested to see if toxicity remains
- If successful, provides clue to source of toxicity

Section 8
Alkalinity



Measurement of Alkalinity for Process Control

Intermediate Wastewater Lab Workshop

Agenda

- Introduction
- Importance in Wastewater Treatment
- Method Summary
- Titration Apparatus
- Required Reagents
- Sampling and Storage
- Interferences

Introduction

- Alkalinity is a general measure of the ionic characteristics of water.
- Others: pH, redox potential, hardness, and conductivity.
- Not normally a compliance-monitoring requirement.

Alkalinity

- Defined as the measurement of a water's capacity to neutralize an acid
- An acid releases H^+
- The alkalinity in the water will absorb H^+
- Most common ions that add alkalinity are OH^-, CO_3^-,HCO_3^-

Importance in Wastewater Treatment

- Chemical and biological treatment systems
- Biological nutrient removal
- Anaerobic digestion control
- Ammonia removal by air stripping

Activated Sludge Alkalinity

- Essential to process control
- Insufficient alkalinity:
  - Reduces organism activity
  - May result in low effluent pH
  - May result in extremely high chlorine demand in disinfection process

Alkalinity Determination

- Titration against a standard acid:
  - Color change of standard indicator
  - pH meter
- Results expressed as total alkalinity, mg/L as calcium carbonate
- Buret Titration Method, *SM*$_{19}$ 2320 B

Alkalinity Determination

- Measured by determining the amount of acid needed to drop the pH of a sample to a certain endpoint
  - Phenolphthalein alkalinity is measured by titrating to a pH of 8.3
  - Total alkalinity is measured by titrating to a pH of 4.5

pH End Points

Sample Composition	End Point pH	
	Total Alkalinity	Phenolphthalein Alkalinity
Alk ~ 30 mg/L	pH 4.9	pH 8.3
Alk ~ 150 mg/L	pH 4.6	pH 8.3
Alk ~ 500 mg/L	pH 4.3	pH 8.3
Silicates or Phosphates present	pH 4.5	pH 8.3
Industrial Waste or Complex System	pH 4.5	pH 8.3
Routine or Automated Process	pH 4.5	pH 8.3

Alkalinity

- Alkalinity caused by OH^- is called
  - *hydroxyl alkalinity*
- Alkalinity caused by CO_3^- is called
  - *carbonate alkalinity*
- Alkalinity caused by HCO_3^- is called
  - *bicarbonate alkalinity*
- The combined effect of all three types is called
  - *total alkalinity*

Apparatus

- Buret and stand
- Beaker, 250 mL
- Stir plate
- Stir bar

Sampling and Storage

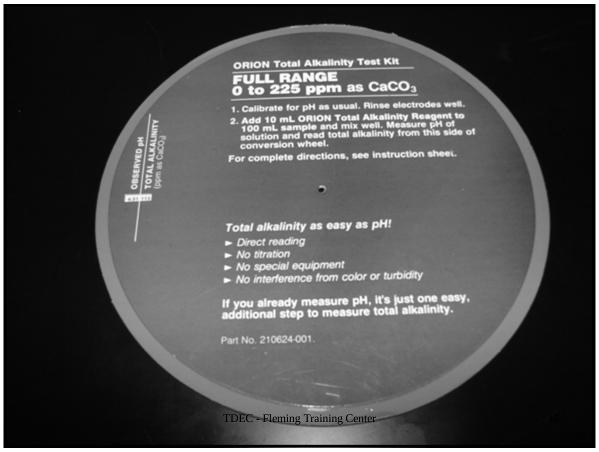
- Collect samples in clean plastic or glass bottles
- Avoid excessive agitation or prolonged exposure to air
- Analyze as soon as possible
  - May be stored for 24 hrs at 4°C
- Warm to room temperature before analysis.

Interferences

- Highly colored or turbid samples may mask the color change at the end point.
  - Use a pH meter for these samples.
- Chlorine may interfere with indicators.
  - Add one drop 0.1N sodium thiosulfate to eliminate this interference.

Orion pH Probe

- Very simple
- No color change to watch
- Must have a properly calibrated pH meter
- By adding a reagent, the determination of alkalinity is made by measuring the drop in pH and comparing the measurement to a calibration chart that show the relationship between pH and alkalinity
- ***Not an approved method.***



ORION Total Alkalinity Test Kit

FULL RANGE
0 to 225 ppm as $CaCO_3$

1. Calibrate for pH as usual. Rinse electrodes well.
2. Add 10 mL ORION Total Alkalinity Reagent to 100 mL sample and mix well. Measure pH of solution and read total alkalinity from this side of conversion wheel.

For complete directions, see instruction sheet.

Total alkalinity as easy as pH!

- *Direct reading*
- *No titration*
- *No special equipment*
- *No interference from color or turbidity*

If you already measure pH, it's just one easy, additional step to measure total alkalinity.

Part No. 210624-001.

	Influent	Effluent
	mL sample used =	mL sample used =
Bench 1	total mL to pH 4.5 =	total mL to pink =
Bench 2	total mL to pink =	total mL to pH 4.5 =
Bench 3	total mL to pH 4.5 =	total mL to pink =
Bench 4	total mL to pink =	total mL to pH 4.5 =
Bench 5a	total mL to pH 4.5 =	total mL to pink =
Bench 5b	total mL to pink =	

Influent Alkalinity - Titrate to pH 4.5 or pink color

$$\text{Alkalinity} = \frac{(\text{ mL titrant used to reach pH of 8.3 })(\text{ 0.02 N } H_2SO_4)(\text{ 50,000 })}{\text{mL sample}}$$

$$\text{Alkalinity} = \frac{(\underline{\qquad\qquad\qquad\qquad})(\text{ 0.02 N } H_2SO_4)(\text{ 50,000 })}{\underline{\qquad\qquad}\text{ mL sample}} =$$

Effluent Alkalinity - Titrate to pH 4.5 or pink color

$$\text{Alkalinity} = \frac{(\text{ total mL titrant used to reach pH of 4.5 })(\text{ 0.02 N } H_2SO_4)(\text{ 50,000 })}{\text{mL sample}}$$

$$\text{Alkalinity} = \frac{(\underline{\qquad\qquad\qquad\qquad})(\text{ 0.02 N } H_2SO_4)(\text{ 50,000 })}{\underline{\qquad\qquad}\text{ mL sample}} =$$

Orion Alkalinity Method =

Alkalinity

DOC316.53.01151

USEPA[1] Buret Titration Method[2] **Method 8221**

(0 to 5,000 mg/L as $CaCO_3$) **Buret Titration**

Scope and Application: For water, wastewater and seawater

[1] USEPA Accepted

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater, 2320 B*

Test preparation

Before starting the test:

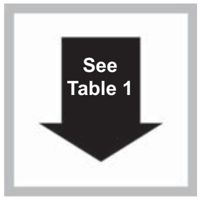
Read the entire procedure before starting the test.
A pH meter is required for NPDES reporting and is recommended for best results.
Substitute six drops of Phenolphthalein Indicator Solution for the Phenolphthalein Indicator Powder Pillow if necessary
Substitute six drops of Bromcresol Green-Methyl Red Indicator Solution for the Bromcresol Green-Methyl Red Powder Pillow if necessary.
Results in mg/L as $CaCO_3$ ÷ 17.12 = grains per gallon

Collect the following items:

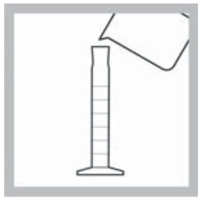
Description	Quantity
Bromcresol Green-Methyl Red indicator powder pillow	1
Phenolphthalein indicator powder pillow	1
Sulfuric acid standard solution, 0.020 N	varies[1]
Buret clamp, double	1
Buret, Class A, 25-mL	1
Graduated cylinder	varies[1]
Erlenmeyer flask, 250-mL	1
Funnel, Micro	1
Support Stand	1

[1] See *Consumables and replacement items* on page 6.

Buret titration (Method 8221)



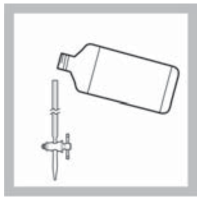
1. Select a sample volume from the *Sample volume selection for expected concentration* table that corresponds to the expected alkalinity concentration in mg/L as calcium carbonate ($CaCO_3$).



2. Use a graduated cylinder or pipet to measure the sample volume.

3. Transfer the sample into a 250-mL Erlenmeyer flask. Dilute to about 50-mL with deionized water if necessary.

4. Add the contents of one Phenolphthalein Indicator Powder Pillow. Swirl to mix. (Omit this step when using a pH meter.)



5. Fill a 25-mL buret to the zero mark with 0.020 N Sulfuric Acid standard solution.

6. While swirling the flask, titrate the sample until the solution color changes from pink to colorless (pH 8.3).

If the solution is colorless before titrating with sulfuric acid, the phenolphthalein alkalinity is zero.

7. Calculate:

mL Titrant × multiplier used = mg/L phenolphthalein alkalinity as $CaCO_3$.

8. Add the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow to the titrated sample. Swirl to mix.

Do not add indicator if a pH meter is used.

Specific sample composition may require titration to a specific pH (see the *Alkalinity relationship* table).

Buret titration (Method 8221)

9. Continue the titration until a light pink end point is reached.

10. Calculate:

mL Titrant × multiplier used = mg/L total alkalinity as $CaCO_3$.

Table 1 Sample volume selection for expected concentration

Range (mg/L as $CaCO_3$)	Sample Volume (mL)	Sulfuric Acid	Multiplier
0–500	50	20353	20
400–1000	25	20353	40
1000–2500	10	20353	100
2000–5000	5	20353	200

The end points in the *Alkalinity endpoints* table are recommended for determining total alkalinity in water samples of various compositions and alkalinity concentrations.

Table 2 Alkalinity endpoints

Sample composition	End point pH	
	Total Alkalinity	Phenolphthalein Alkalinity
Alkalinity about 30 mg/L	pH 4.9	pH 8.3
Alkalinity about 150 mg/L	pH 4.6	pH 8.3
Alkalinity about 500 mg/L	pH 4.3	pH 8.3
Silicates or phosphates present	pH 4.5	pH 8.3
Industrial wastes or complex system	pH 4.5	pH 8.3
Routine or Automated Analyses	pH 4.5	pH 8.3

Total alkalinity primarily includes hydroxide, carbonate, and bicarbonate alkalinities. The concentration of these types in a sample may be determined when the phenolphthalein and total alkalinities are known (*Alkalinity relationship* table).

Table 3 Alkalinity relationship

Row	Result of Titration	Hydroxide Alkalinity Equals:	Carbonate Alkalinity Equals:	Bicarbonate Alkalinity Equals:
1	Phenolphthalein Alkalinity equal to 0	0	0	Total Alkalinity
2	Phenolphthalein Alkalinity equal to Total Alkalinity	Total Alkalinity	0	0
3	Phenolphthalein Alkalinity less than one-half of Total Alkalinity	0	Phenolphthalein Alkalinity times 2	Total Alkalinity minus two times Phenolphthalein Alkalinity
4	Phenolphthalein Alkalinity equal to one-half of Total Alkalinity	0	Total Alkalinity	0
5	Phenolphthalein Alkalinity greater than one-half of Total Alkalinity	2 times Phenolphthalein Alkalinity minus Total Alkalinity	2 times the difference between Total and Phenolphthalein Alkalinity	0

Use the *Alkalinity relationship* table with the following procedure:

1. Does the phenolphthalein alkalinity equal zero? If yes, use Row 1.
2. Does the phenolphthalein alkalinity equal total alkalinity? If yes, use Row 2.
3. Divide the total alkalinity by 2 to calculate one-half the total alkalinity.
4. Select Row 3, 4 or 5 based on comparing the result of step c (one-half total alkalinity) with the phenolphthalein alkalinity.
5. Perform the required calculations if any.
6. Check your results. The sum of the three alkalinity types will equal the total alkalinity.

Example:
A sample has 170 mg/L as $CaCO_3$ phenolphthalein alkalinity and 250 mg/L as CaCO3 total alkalinity. What is the concentration of hydroxide, carbonate, and bicarbonate alkalinities?

- **a.** The phenolphthalein alkalinity does not equal zero but 170 mg/L.
- **b.** The phenolphthalein alkalinity does not equal total alkalinity (170 mg/L vs. 250 mg/L).
- **c.** One-half of the total alkalinity equals 125 mg/L.
- **d.** Because the phenolphthalein alkalinity of 170 mg/L is greater than one-half the total alkalinity of 125 mg/L, select Row 5.

The hydroxide alkalinity is equal to:

$$2 \times 170 = 340$$

$$340 - 250 = 90 \text{ mg/L hydroxide alkalinity}$$

The carbonate alkalinity is equal to:

$$250 - 170 = 80$$

$$80 \times 2 = 160 \text{ mg/L carbonate alkalinity}$$

The bicarbonate alkalinity is equal to zero mg/L.

Check:

90 mg/L hydroxide alkalinity + 160 mg/L carbonate alkalinity + 0 mg/L bicarbonate alkalinity = 250 mg/L

The answer is correct.

The sum of each type equals the total alkalinity (250 mg/L).

Interferences

Chlorine at levels above 3.5 mg/L cause a yellow-brown color upon the addition of the Bromcresol Green-Methyl Red Indicator Powder Pillow. Residual chlorine interference with the indicator may be removed by adding a drop of 0.1 N Sodium Thiosulfate Standard Solution* before adding the indicator.

Highly colored or turbid samples may mask the color change at the end point. Use a pH meter for these samples, titrating to pH 8.3 for phenolphthalein alkalinity and the appropriate pH (see the *Alkalinity endpoints* table) for total alkalinity.

Sampling and storage

Collect samples in plastic or glass bottles. Fill completely and cap tightly. Avoid excessive agitation and prolonged exposure to air. Samples should be analyzed as soon as possible after collection but can be stored at least 24 hours by cooling to 4 °C (39 °F) or below. Warm to room temperature before analyzing.

Accuracy check

End point confirmation

- To accurately determine the phenolphthalein alkalinity end point, mix the contents of one Phenolphthalein Indicator Powder Pillow and the contents of one pH 8.3 Buffer Powder Pillow with 50 mL of deionized water in a 250-mL Erlenmeyer flask. The resulting color is the end point.
- To accurately determine the total alkalinity end point, mix the contents of one pH 4.5 Buffer Powder Pillow and the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow with 50 mL of deionized water in a 250-mL Erlenmeyer flask. Titrate to a light pink color change.

Standard additions method (Sample Spike)

Perform the standard additions method check as follows:

1. Break the top off an Alkalinity Voluette® Ampule Standard Solution, 0.500 N.
2. Use the TenSette Pipet* to add 0.1 mL of standard to the sample titrated in step 6 or step 9. Resume titration back to the same end point. Record the volume of titrant needed.
3. Repeat, using two more additions of 0.1 mL. Titrate to the end point after each addition.
4. The mL of titrant required should increase by 2.5 mL for each 0.1 mL increment of standard added.

Summary of method

Alkalinity is expressed as P (phenolphthalein) alkalinity or as T (total) alkalinity. Both types are determined by titration with a Sulfuric Acid Standard Solution to an end point evidenced by the color change of an indicator solution or determined with a pH meter. The P alkalinity is determined by titration to a pH of 8.3 and registers the total hydroxide and one half the carbonate present. The T alkalinity is determined by titration to a pH of 4.5. The total alkalinity includes all carbonate, bicarbonate and hydroxide alkalinity. Alternatively, total alkalinity end points may be determined by using a pH meter and titrating to the specific pH required for the sample composition.

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* See *Consumables and replacement items* on page 6.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Catalog number
Bromcresol Green-Methyl Red Indicator Powder Pillows	1 pillow	100/pkg	94399
Phenolphthalein Indicator Powder Pillows	1 pillow	100/pkg	94299
Sulfuric Acid Standard Solution, 0.020 N	varies	1 L	20353

Required apparatus

Description	Quantity/test	Unit	Catalog number
Buret Clamp, double	1	each	32800
Buret, Class A, 25-mL	1	each	2636540
Select one or more based on sample volume:			
Cylinder, graduated, 5-mL	—	each	50837
Cylinder, graduated, 10-mL	—	each	50838
Cylinder, graduated, 25-mL	—	each	50840
Cylinder, graduated, 50-mL	—	each	50841
Flask, Erlenmeyer, 250-mL	1	each	50546
Pipet, volumetric, Class A, 5-mL,	—	each	1451537
Pipet, volumetric, Class A, 10-mL	—	each	1451537
Pipet Filler, Safety Bulb	—	each	1465100
Ampule Breaker	—	each	2196800
Funnel, Micro	1	each	2584335
Support Stand	1	each	56300

Required standards

Description	Unit	Catalog number
Alkalinity Standard Solution, Voluette® Ampules, 0.500 N, 10-mL	16/pkg	1427810
Buffer Powder Pillows, pH 4.5	25/pkg	89568
Buffer Powder Pillows, pH 8.3	25/pkg	89868
Water, deionized	4 L	27256

Optional items

Description	Unit	Catalog number
Sodium Thiosulfate Standard Solution, 0.1 N	—	32332
TenSette Pipet, 0.1–1.0 mL	—	1970001
Tips for Tensette Pipet	50/pkg	2185696

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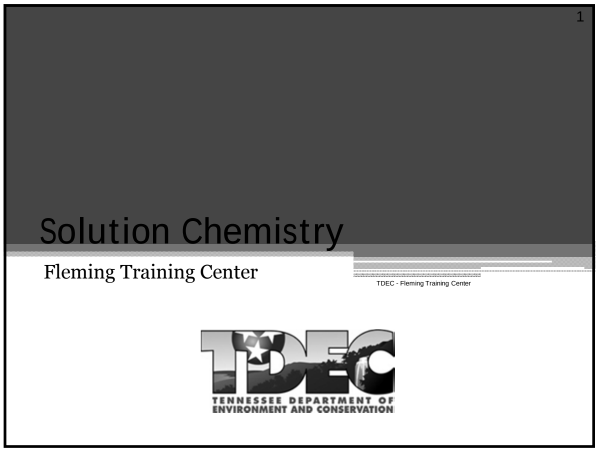
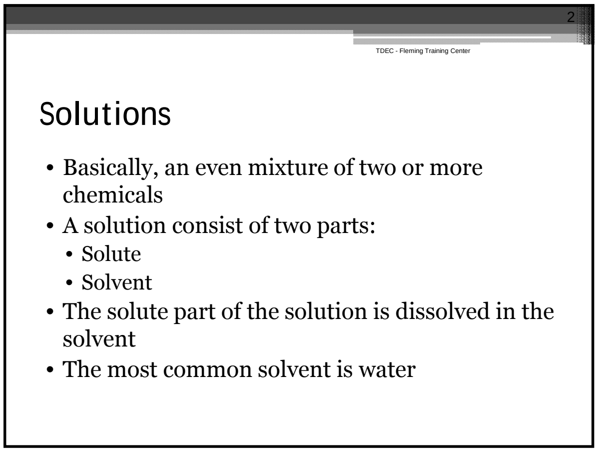
Updated February 2008, Edition 5

Section 9
Solutions



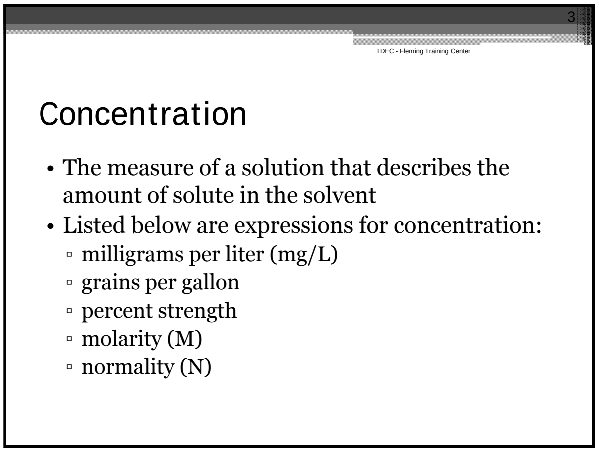
Solution Chemistry

Fleming Training Center



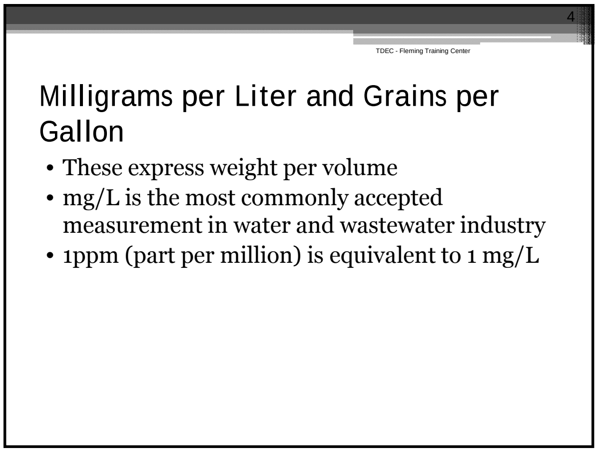
Solutions

- Basically, an even mixture of two or more chemicals
- A solution consist of two parts:
  - Solute
  - Solvent
- The solute part of the solution is dissolved in the solvent
- The most common solvent is water



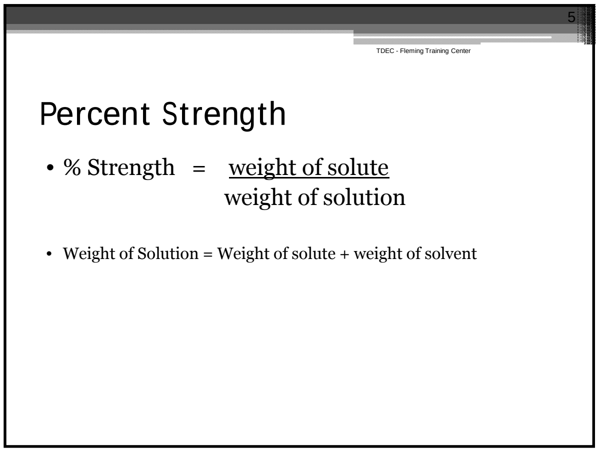
Concentration

- The measure of a solution that describes the amount of solute in the solvent
- Listed below are expressions for concentration:
  - milligrams per liter (mg/L)
  - grains per gallon
  - percent strength
  - molarity (M)
  - normality (N)



Milligrams per Liter and Grains per Gallon

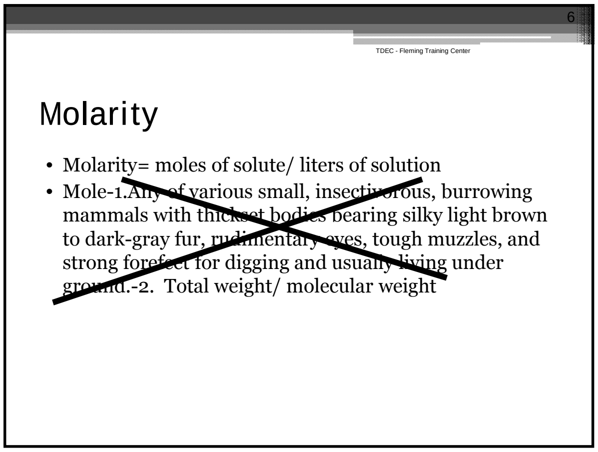
- These express weight per volume
- mg/L is the most commonly accepted measurement in water and wastewater industry
- 1ppm (part per million) is equivalent to 1 mg/L



Percent Strength

- % Strength = $\frac{\text{weight of solute}}{\text{weight of solution}}$

- Weight of Solution = Weight of solute + weight of solvent



Molarity

- Molarity= moles of solute/ liters of solution
- Mole-1.~~Any of various small, insectivorous, burrowing mammals with thickset bodies bearing silky light brown to dark-gray fur, rudimentary eyes, tough muzzles, and strong forefeet for digging and usually living under ground.~~-2. Total weight/ molecular weight

Normality

- Normality = $\frac{\text{number of equivalent weights of solute}}{\text{liter of solution}}$
- Number of equivalent weights = $\frac{\text{total weight of solute}}{\text{equivalent weight}}$
- Equivalent weight = $\frac{\text{molecular weight}}{\text{number of positive charges}}$

Molarity and Normality

- How many grams of Na_2CO_3 would it take to make 1 L of a 1 molar and a 1 normal solution?
  - Step 1 - Determine molecular weight:
    - Na = 22.99 amu x 2 = 45.98 amu
    - C = 12.01 amu x 1 = 12.01 amu
    - O = 16.00 amu x 3 = 48.00 amu

    Molecular weight = 105.99 amu
  - $Na_2CO_3 \rightarrow Na^+ + Na^+ + CO_3^{2-}$

Molarity

grams/mole = molecular weight

- Step 2 - Calculate the grams required
  - grams required = (#moles required)(g/mole)
  - grams required = (1 mole)(105.99 g/mole)
  - grams required = 105.99 g
- Step 3 - Calculate molarity of the solution
  - Molarity = $\frac{\text{\# moles}}{\text{volume of solvent}}$
  - Molarity = $\frac{\text{1 mole}}{\text{1 liter of water}}$
  - Molarity = 1M
- Every 105.99 grams of Na_2CO_3 in 1L of water gives a 1M solution

Molarity Shortcut

- Grams required=

Na = 22.99 amu x 2 = 45.98 amu
C = 12.01 amu x 1 = 12.01 amu
O = 16.00 amu x 3 = 48.00 amu
Molecular weight = 105.99 amu

$Na_2CO_3 \rightarrow Na^+ + Na^+ + CO_3^{2-}$

(Molarity needed)(molecular wt)(L sol'n)

(1M)(105.99)(1 L)

105.99 g/L = 1 M solution

Normality

- Step 2 - Calculate the equivalent weight
  - Equivalent weight = $\frac{\text{molecular weight}}{\text{\# of (+) charges}}$
  - Equivalent weight = $\frac{105.99}{2}$
  - Equivalent weight = 53 grams

Normality

- Step 2b - Calculate the number of equivalent weights
  - #Equivalent weights = $\frac{\text{total weight}}{\text{equivalent weights}}$
  - #Equivalent weights = $\frac{\text{105.99 g}}{\text{53 g/equivalent}}$
  - #Equivalent weights = 2

Normality

- OR Step 2b - Calculate the grams required
  - g required = (#equivalent weights)(g/equivalent wt)
  - g required = (2 equivalent wts)(53g/equivalent wt)
  - g required = 106 g

Normality

- Step 3 - Calculate Normality
  - Normality = $\frac{\text{\# of equivalents}}{\text{volume of solvent}}$
  - Normality = $\frac{\text{2 equivalents}}{\text{1 L of water}}$
  - Normality = 2N
- Every 105.99 grams of Na_2CO_3 in 1L of water gives a 2N solution
  - to make a 1N solution, cut the grams in half

Normality Shortcut

- Grams required =

Na	=	22.99 amu x 2	=	45.98 amu
C	=	12.01 amu x 1	=	12.01 amu
O	=	16.00 amu x 3	=	48.00 amu
		Molecular weight	=	105.99 amu

$Na_2CO_3 \rightarrow Na^+ + Na^+ + CO_3^{2-}$

$$\frac{\text{(Normality needed)(molecular weight)(L sol'n)}}{\text{\# of positive charges}}$$

$$\frac{(1\text{ N})(105.99)(1\text{ L})}{2}$$

53 g/L = 1N solution

Dilutions

- Weakening a stock solution
- Start with a stock solution, add an amount to the volumetric flask, and fill to mark
- Can use the following calculation with any expression of concentration or normality

Use this equation to make calculation:

$C_1V_1 = C_2V_2$

$N_1V_1 = N_2V_2$

1 means stock or initial concentration or volume

2 is the concentration or volume you end up with

Dilution Examples

- Operator has 12% bleach on hand and wants to make 25 gallons of 8%.

$C_1V_1 = C_2V_2$

$(0.12)(V_1) = (0.08)(25\text{ gal})$

$V_1 = \frac{(0.08)(25\text{ gal})}{0.12}$

$V_1 = 16.7\text{ gals}$

Dilution Examples

- Operator has 100 mL of 5N sulfuric acid on hand and wants to make 1N. How much water should be added?

$N_1V_1 = N_2V_2$

$(5\text{N})(100\text{ mL}) = (1\text{N})(V_2)$

$\frac{(5\text{N})(100\text{ mL})}{1\text{ N}} = (V_2)$

$500\text{ mL} = V_2$

500 mL is final volume, you started with 100 mLs and added 400 mL to get a final volume of 500 mL

Periodic Table of the Elements

KEY

Atomic Mass → 12.011 –4 +2 +4 ← Selected Oxidation States
Symbol → **C**
Atomic Number → **6**
Electron Configuration → 2-4

Relative atomic masses are based on ^{12}C = 12.000

Note: Mass numbers in parentheses are mass numbers of the most stable or common isotope.

Period	Group 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.00794 +1 –1 **H** **1** 1																	4.00260 0 **He** **2** 2
2	6.941 +1 **Li** **3** 2-1	9.01218 +2 **Be** **4** 2-2											10.81 +3 **B** **5** 2-3	12.0111 –4 +2 +4 **C** **6** 2-4	14.0067 –3 –2 –1 +1 +2 +3 +4 +5 **N** **7** 2-5	15.9994 –2 **O** **8** 2-6	18.998403 –1 **F** **9** 2-7	20.179 0 **Ne** **10** 2-8
3	22.98977 +1 **Na** **11** 2-8-1	24.305 +2 **Mg** **12** 2-8-2											26.98154 +3 **Al** **13** 2-8-3	28.0855 –4 +2 +4 **Si** **14** 2-8-4	30.97376 –3 +3 +5 **P** **15** 2-8-5	32.06 –2 +4 +6 **S** **16** 2-8-6	35.453 –1 +1 +3 +5 +7 **Cl** **17** 2-8-7	39.948 0 **Ar** **18** 2-8-8
4	39.0983 +1 **K** **19** 2-8-8-1	40.08 +2 **Ca** **20** 2-8-8-2	44.9559 +3 **Sc** **21** 2-8-9-2	47.88 +2 +3 +4 **Ti** **22** 2-8-10-2	50.9415 +2 +3 +4 +5 **V** **23** 2-8-11-2	51.996 +2 +3 +6 **Cr** **24** 2-8-13-1	54.9380 +2 +3 +4 +7 **Mn** **25** 2-8-13-2	55.847 +2 +3 **Fe** **26** 2-8-14-2	58.9332 +2 +3 **Co** **27** 2-8-15-2	58.69 +2 +3 **Ni** **28** 2-8-16-2	63.546 +1 +2 **Cu** **29** 2-8-18-1	65.39 +2 **Zn** **30** 2-8-18-2	69.72 +3 **Ga** **31** 2-8-18-3	72.59 –4 +2 +4 **Ge** **32** 2-8-18-4	74.9216 –3 +3 +5 **As** **33** 2-8-18-5	78.96 –2 +4 +6 **Se** **34** 2-8-18-6	79.904 –1 +1 +5 **Br** **35** 2-8-18-7	83.80 0 +2 **Kr** **36** 2-8-18-8
5	85.4678 +1 **Rb** **37** 2-8-18-8-1	87.62 +2 **Sr** **38** 2-8-18-8-2	88.9059 +3 **Y** **39** 2-8-18-9-2	91.224 +4 **Zr** **40** 2-8-18-10-2	92.9064 +3 +5 **Nb** **41** 2-8-18-12-1	95.94 +3 +6 **Mo** **42** 2-8-18-13-1	(98) +4 +6 +7 **Tc** **43** 2-8-18-14-1	101.07 +3 **Ru** **44** 2-8-18-15-1	102.906 +3 **Rh** **45** 2-8-18-16-1	106.42 +2 +4 **Pd** **46** 2-8-18-18	107.868 +1 **Ag** **47** 2-8-18-18-1	112.41 +2 **Cd** **48** 2-8-18-18-2	114.82 +3 **In** **49** 2-8-18-18-3	118.71 +2 +4 **Sn** **50** 2-8-18-18-4	121.75 –3 +3 +5 **Sb** **51** 2-8-18-18-5	127.60 –2 +4 +6 **Te** **52** 2-8-18-18-6	126.905 –1 +1 +5 +7 **I** **53** 2-8-18-18-7	131.29 0 +2 +4 +6 **Xe** **54** 2-8-18-18-8
6	132.905 +1 **Cs** **55** 2-8-18-18-8-1	137.33 +2 **Ba** **56** 2-8-18-18-8-2	138.906 +3 **La** **57** 2-8-18-18-9-2	178.49 +4 **Hf** **72** **18-32-10-2	180.948 +5 **Ta** **73** -18-32-11-2	183.85 +6 **W** **74** -18-32-12-2	186.207 +4 +6 +7 **Re** **75** -18-32-13-2	190.2 +3 +4 **Os** **76** -18-32-14-2	192.22 +3 +4 **Ir** **77** -18-32-15-2	195.08 +2 +4 **Pt** **78** -18-32-17-1	196.967 +1 +3 **Au** **79** -18-32-18-1	200.59 +1 +2 **Hg** **80** -18-32-18-2	204.383 +1 +3 **Tl** **81** -18-32-18-3	207.2 +2 +4 **Pb** **82** -18-32-18-4	208.980 +3 +5 **Bi** **83** -18-32-18-5	(209) +2 +4 **Po** **84** -18-32-18-6	(210) **At** **85** -18-32-18-7	(222) 0 **Rn** **86** -18-32-18-8
7	(223) +1 **Fr** **87** -18-32-18-8-1	226.025 +2 **Ra** **88** -18-32-18-8-2	227.028 +3 **Ac** **89** -18-32-18-9-2	(261) **Rf** **104**	(262) **Db** **105**	(263) **Sg** **106**	(264) **Bh** **107**	(265) **Hs** **108**	(268) **Mt** **109**	(269) **Uun**	(272) **Uuu** **111**	(277) **Uub** **112**		(285) **Uuq** **114**				

**Denotes the presence of (2-8-) for elements 72 and above

*The systematic names and symbols for elements of atomic numbers above 109 will be used until the approval of trivial names by IUPAC.

140.12 +3 +4 **Ce** **58**	140.908 +3 **Pr** **59**	144.24 +3 **Nd** **60**	(145) +3 **Pm** **61**	150.36 +2 +3 **Sm** **62**	151.96 +2 +3 **Eu** **63**	157.25 +3 **Gd** **64**	158.925 +3 **Tb** **65**	162.50 +3 **Dy** **66**	164.930 +3 **Ho** **67**	167.26 +3 **Er** **68**	168.934 +3 **Tm** **69**	173.04 +2 +3 **Yb** **70**	174.967 +3 **Lu** **71**
232.038 +4 **Th** **90**	231.036 +4 +5 **Pa** **91**	238.029 +3 +4 +5 +6 **U** **92**	237.048 +3 +4 +5 +6 **Np** **93**	(244) +3 +4 +5 +6 **Pu** **94**	(243) +3 +4 +5 +6 **Am** **95**	(247) +3 **Cm** **96**	(247) +3 +4 **Bk** **97**	(251) +3 **Cf** **98**	(252) **Es** **99**	(257) **Fm** **100**	(258) **Md** **101**	(259) **No** **102**	(260) **Lr** **103**

Common Valences

1+

Ammonium, NH_4^+
Cuprous, Cu^+
Hydrogen, $H+$
Hydronium, H_3O^+
Potassium, K^+
Silver, Ag^+
Sodium, Na^+

2+

Barium, Ba^{2+}
Calcium, Ca^{2+}
Cupric, Cu^{2+}
Ferrous, Fe^{2+}
Lead, Pb^{2+}
Magnesium, Mg^{2+}
Mercuric, Hg^{2+}
Nickel, Ni^{2+}
Zinc, Zn^{2+}

3+

Aluminum, Al^{3+}
Chromic, Cr^{3+}
Ferric, Fe^{3+}

1-

Acetate, $C_2H_3O_2^-$
Bicarbonate, HCO_3^-
Bromide, Br^-
Chlorate, ClO_3^-
Chloride, Cl^-
Hydroxide, OH^-
Iodide, I^-
Nitrate, NO_3^-
Nitrite, NO_2^-
Bisulfate, HSO_4^-

2-

Carbonate, CO_3^{2-}
Chromate, CrO_4^{2-}
Peroxide, O_2^{2-}
Sulfate, SO_4^{2-}
Sulfide, S^{2-}
Sulfite, SO_3^{2-}

3-

Phosphate, PO_4^{3-}

Intermediate Wastewater Laboratory Workshop
Laboratory Solutions

1. A laboratory solution is made using 52 milligrams of sodium chloride (NaCl) dissolved in 1-liter volumetric flask filled to the mark. What is the mg/L concentration of the solution?

2. If 33 pounds of a chemical is added to 148 pounds of water, what is the % strength by weight?

3. You are given 100 mL of 2.8N HCl. How many mL of water should be added to make 0.4N HCl?

4. 250 mL of 3N NaOH is diluted to 1000 mL. What is the new normality of the solution?

5. 500 mL of 10N NaOH is diluted to 1 liter. What is the new normality of the solution?

6. You are given 20 mL of 30N HCl. How many mL of water should be added to make 1.1N HCl?

7. An operator needs a 0.1N solution in order to conduct an analysis. The operator has a 1.5N solution on hand. How much (mL) of the 1.5N solution is needed to make 1L of 0.1N solution?

8. An operator needs a 0.1N solution in order to conduct an analysis. The operator has 2.0N solution on hand. How many milliliters of the 2.0N solution is needed to make one liter of 0.1N solution?

9. 450 mL of 5N NaOH is diluted to 1L. What is the new normality of the solution?

10. You are given 8 mL of 15N H_2SO_4. How much water (in mL) should be added to make 0.4N H_2SO_4?

11. An operator needs a 0.2N solution in order to conduct analysis. The operator has 2.5N solution on hand. How many mL of the 2.5N solution is needed to make one-half liter of 0.2N solution?

12. An operator needs to make 1-liter of a 1N and a 1M solution of sodium bicarbonate ($NaHCO_3$). How many grams would be needed for each? (Hint: bicarbonate = HCO_3)

13. An operator needs to make 1-liter of a 1N and a 1M solution of sodium hydroxide (NaOH). How many grams would be needed for each?

14. An operator needs to make ½ -liter of a 5N and a 5M solution of ferric sulfate $Fe_2(SO_4)_3$. How many grams would be needed for each?

Answers

1. 52 mg/L
2. 18.2%
3. 600 mL to add
4. 0.75N
5. 5N
6. 525.5 mL to add
7. 66.67 mL
8. 50 mL
9. 2.25N
10. 292 mL to add
11. 40 mL
12. 84.01 g for 1M
    84.01 g for 1N
13. 40 g for 1M
    40 g for 1N
14. 999.78 g for 5M
    166.7 g for 5N

Intermediate Wastewater Laboratory Workshop
Laboratory Solutions

1. A laboratory solution is made using 52 milligrams of sodium chloride (NaCl) dissolved in 1-liter volumetric flask filled to the mark. What is the mg/L concentration of the solution?

$$\frac{52\text{ mg}}{1\text{ L}} = \boxed{52\text{ mg/L}}$$

2. If 33 pounds of a chemical is added to 148 pounds of water, what is the % strength by weight?

$$\%\text{ strength} = \frac{\text{weight of chemical, lbs}}{\text{wt. of water} + \text{wt. of chem.}} \times 100$$

$$= \frac{33}{33+148} \times 100 = \frac{33}{181} \times 100 = \boxed{18.2\%}$$

3. You are given $\overset{V_1}{100}$ mL of $\overset{C_1}{2.8}$N HCl. How many mL of water should be added to make $\overset{C_2}{0.4}$N HCl?

$$(\text{Conc}_1)(\text{Vol}_1) = (\text{Conc}_2)(\text{Vol}_2) \qquad 700 - 100 = \boxed{600\text{ mL}}$$

$$(2.8)(100) = (0.4)(V_2)$$

$$\frac{280}{0.4} = 700\text{ mL} = V_2$$

4. $\overset{V_1}{250}$ mL of $\overset{C_1}{3}$N NaOH is diluted to $\overset{V_2}{1000}$ mL. What is the new normality of the solution?

$$(250)(3) = (C_2)(1000)$$

$$\frac{750}{1000} = C_2 = \boxed{0.75\text{ N}}$$

V_1 C_1 V_2

5. 500 mL of 10N NaOH is diluted to 1 liter. What is the new normality of the solution? 1L = 1000mL

$$(500)(10) = (C_2)(1000)$$

$$\frac{5000}{1000} = C_2 = \boxed{5\text{ N}}$$

V_1 C_1

6. You are given 20 mL of 30N HCl. How many mL of water should be added to make 1.1N HCl?

C_2

$$545 - 20 = \boxed{525\text{ mL}}$$

$$(20)(30) = (V_2)(1.1)$$

$$\frac{600}{1.1} = V_2 = 545\text{ mL}$$

7. An operator needs a 0.1N solution in order to conduct an analysis. The operator has a 1.5N solution on hand. How much (mL) of the 1.5N solution is needed to make 1L of 0.1N solution?

C_1 V_2 C_2

$$(V_1)(1.5) = (1000)(0.1)$$

$$V_1 = \frac{100}{1.5} = \boxed{66.7\text{ mL}}$$

8. An operator needs a 0.1N solution in order to conduct an analysis. The operator has 2.0N solution on hand. How many milliliters of the 2.0N solution is needed to make one liter of 0.1N solution?

C_1 V_2 C_2

$$(2.0)(V_1) = (1000)(0.1)$$

$$V_1 = \frac{100}{2.0} = \boxed{50\text{ mL}}$$

V_1 C_1 V_2

9. 450 mL of 5N NaOH is diluted to 1L. What is the new normality of the solution?

$$(450)(5) = (1000)(C_2)$$

$$\frac{2250}{1000} = C_2 = \boxed{2.25\text{ N}}$$

V_1 C_1

10. You are given 8 mL of 15N H_2SO_4. How much water (in mL) should be added to make 0.4N H_2SO_4?

C_2

$$(8)(15)=(0.4)(V_2)$$

$$\frac{120}{0.4}=V_2=300\text{ mL}$$

$$300-8=\boxed{292\text{ mL}}$$

11. An operator needs a 0.2N solution in order to conduct analysis. The operator has 2.5N solution on hand. How many mL of the 2.5N solution is needed to make one-half liter of 0.2N solution? C_1

V_2 C_2

$$(V_1)(2.5)=(500)(0.2)$$

$$V_1=\frac{100}{2.5}=\boxed{40\text{ mL}}$$

12. An operator needs to make 1-liter of a 1N and a 1M solution of sodium bicarbonate ($NaHCO_3$). How many grams would be needed for each? (Hint: bicarbonate = HCO_3)

$Na^+\ HCO_3^-$

Na → 22.98977 x 1 = 22.99
H → 1.00794 x 1 = 1.01
C → 12.0111 x 1 = 12.01
O → 15.9994 x 3 = + 48.00
84.01

Molarity → grams needed = (molarity needed)(molecular wt.)(L sol'n)

$$=(1\text{M})(84)(1\text{L})=\boxed{84\text{ g}}$$

Normality → grams needed = $\dfrac{\text{(normality needed)(molecular wt.)(L sol'n)}}{\text{\# of positives}}$

$$=\frac{(1\text{N})(84)(1\text{L})}{1}=\boxed{84\text{ g}}$$

13. An operator needs to make 1-liter of a 1N and a 1M solution of sodium hydroxide (NaOH). How many grams would be needed for each?

Na → 22.98977 $Na^+ OH^-$
O → 15.9994
H → 1.00794
39.99711

molarity = $(1M)(40)(1L) = \boxed{40g}$

normality = $\frac{(1N)(40)(1L)}{1} = \boxed{40g}$

14. An operator needs to make ½ -liter of a 5N and a 5M solution of ferric sulfate $Fe_2(SO_4)_3$. How many grams would be needed for each?

Fe → 55.847 x 2 = 111.694 $Fe_2^{-3} (SO_4)_3^{-2}$
S → 32.06 x 3 = 96.18
O → 15.9994 x 12 = 191.9928
399.8668

molarity = $(5M)(399.8668)(.5L) = \boxed{999.7g}$

normality = $\frac{(5N)(399.8668)(0.5L)}{6} = \boxed{166.6\ g}$

Answers

1. 52 mg/L
2. 18.2%
3. 600 mL to add
4. 0.75N
5. 5N
6. 525.5 mL to add
7. 66.67 mL
8. 50 mL
9. 2.25N
10. 292 mL to add
11. 40 mL
12. 84.01 g for 1M
    84.01 g for 1N
13. 40 g for 1M
    40 g for 1N
14. 999.78 g for 5M
    166.7 g for 5N

Section 10
Chemical Oxygen Demand

Chemical Oxygen Demand (COD)

Approved Methods Under
the Clean Water Act (NPDES)

Why Test For COD??

- To measure the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant.

Sample Collection

- Plastic or glass bottles
- Bottles must be thoroughly cleaned
- Representative sample (100 mL)
- Composite sample preserve at 4°C
- Analyze as soon as possible (same day) OR
- Preserve with H_2SO_4 to pH<2 and cool to 4° C
- Holding time is 28 days after preservation

Chemical Oxygen Demand vs. Biochemical Oxygen Demand

- COD is a good estimate of the first-stage oxygen demand for most municipal wastewaters.
- COD measures the strength of the organic waste that is too toxic for BOD.
- COD results are quicker.
- COD results can be used to estimate BOD.

COD vs BOD (Disadvantages)

- Does not measure the rate of biodegradability.
- Cannot be used to predict the effects of an effluent on the DO in receiving waters OR
- Treatment of a particular wastewater by biological processes.

Watertown, TN

Summary of Method

- The method oxidizes organic substances in the wastewater sample using:
  - Potassium dichromate in 50% sulfuric acid
  - Silver sulfate (catalyst)
  - Mercuric sulfate (remove interferences)
  - Sample heated or reflux for 2 hours

Selection of Method

- Titrimetric (blue-green to reddish):
  - EPA Method 410.1, 410.2 & 410.3
  - SM 5220C (18th, 19th, & 20th editions)
- Colorimetric (manual or automated):
  - EPA Method 410.4
  - SM 5220D (18th, 19th, & 20th editions)
  - Hach Method 8000

Laboratory Apparatus

- Titrimetric Method:
  - Reflux ground-glass apparatus
  - Heat source
  - Buret
- Colorimetric Method:
  - Digestion tubes
  - Hot block (150°C)
  - Spectrometer 420nm and/or 600 nm

Calculations

- Titrimetric
  - $\text{COD, mg/L} = \frac{(A-B)N \times 8000}{S}$
    - A = mL of titrant for blank
    - B = mL of titrant for sample
    - N = Normality of titrant
    - S = mL of sample used

Calculations (continued)

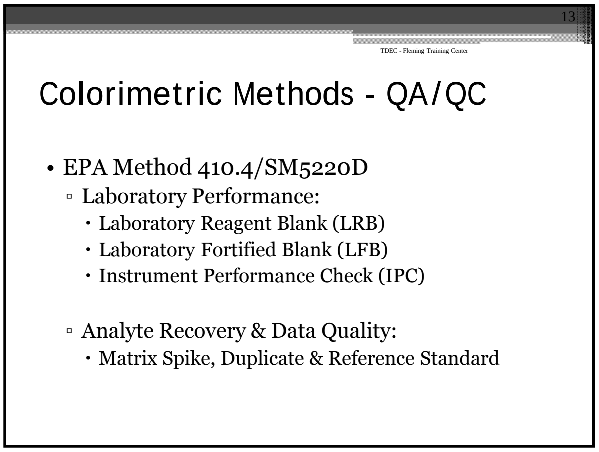
- Colorimetric:
  - Direct reading in mg/L from instrument
  - If different sample volumes used then,
  - $\text{COD, mg/L} = \frac{(\text{mg } O_2 \text{ in final vol.})(1000)}{\text{mL sample}}$

Quality Assurance/Quality Control

- Titrimetric Method EPA 410.2 & 410.3:
  - DI Blank
  - Chloride check
  - Standardize Titrant
  - $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$
- Hach Method 8000:
  - Pre-programmed calibration curve
  - Accuracy Check Standard (potassium acid phthalate)
  - DI Blank

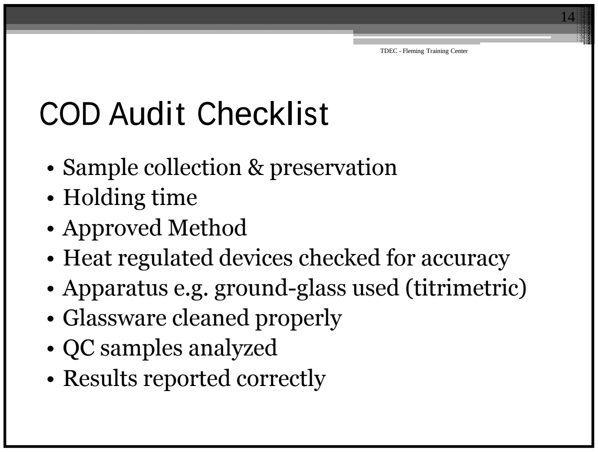
Colorimetric Methods - QA/QC

- EPA Method 410.4/SM5220D
  - Initial Demonstration of Performance:
    - Linear Calibration Range (LCR)
    - Quality Control Sample (QCS)
    - Method Detection Limit (MDL)



Colorimetric Methods - QA/QC

- EPA Method 410.4/SM5220D
  - Laboratory Performance:
    - Laboratory Reagent Blank (LRB)
    - Laboratory Fortified Blank (LFB)
    - Instrument Performance Check (IPC)
  - Analyte Recovery & Data Quality:
    - Matrix Spike, Duplicate & Reference Standard



COD Audit Checklist

- Sample collection & preservation
- Holding time
- Approved Method
- Heat regulated devices checked for accuracy
- Apparatus e.g. ground-glass used (titrimetric)
- Glassware cleaned properly
- QC samples analyzed
- Results reported correctly

Oxygen Demand, Chemical

DOC316.53.01099

USEPA[1] Reactor Digestion Method[2]

Method 8000

0.7 to 40.0[3] mg/L COD; 3 to 150 mg/L COD;
20 to 1500 mg/L COD; 200 to 15,000 mg/L COD

Scope and Application: For water, wastewater; digestion is required

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test

Table 1 Instrument-specific information

Instrument	Light shield	Adapter
DR 5000	—	—
DR 2800	LZV646	—
DR 2700	LZV646	—
DR/2500	—	—
DR/2400	—	5945700

Before starting the test:

DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before performing this test.
DR 2700 and DR/2400: Ultra low range (0.7 to 40.0 mg/L) is not available.
Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidentally misused. Please read all warnings and associated MSDS sheets.
Run one blank with each set of samples. Run all tests (the samples and the blank) with the same lot of vials. The lot number appears on the container label. See *Blanks for colorimetric determination*.
Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Be prepared to wash spills with running water.
Wear appropriate eye protection and clothing for adequate user protection. If contact occurs, flush the affected area with running water. Review and follow reagent MSDS safety instructions carefully.
If high chloride samples are being tested, refer to the Alternate reagents section.

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[1] Ranges 3 to 150 mg/L COD and 20 to 1500 mg/L COD are USEPA approved for wastewater analyses (Standard Method 5220 D), *Federal Register*, April 21, 1980, 45(78), 26811-26812.

[2] Jirka, A.M.; Carter, M.J., *Analytical Chemistry*, 1975, 47(8), 1397

[3] The ULR range is not available on the DR 2700 or the DR/2400.

Collect the following items:

Description	Quantity
Beaker, 250-mL	1
Blender	1
COD Digestion Reagent vials	varies
DRB200 Reactor	1
Light Shield or adapter (see *Instrument-specific information*)	1
Magnetic stirrer and stir bar	1
Opaque shipping container for storage of unused, light-sensitive reagent vials	varies
Pipet, TenSette®, 0.1 to 1.0 mL, with tips (for 200–15,000 mg/L range)	1
Pipet, volumetric, 2.00 mL	2
Pipet Filler, safety bulb	1
Test Tube Rack	2

See *Consumables and replacement items* for reorder information.

Reactor digestion procedure

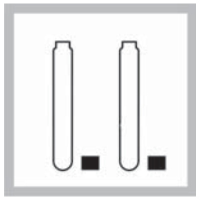
1. Homogenize 100 mL of sample for 30 seconds in a blender. For samples containing large amounts of solids, increase the homogenization time.

If the sample does not contain suspended solids, omit steps 1 and 2.

2. For the 200–15,000 mg/L range or to improve accuracy and reproducibility of the other ranges, pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate.

3. Turn on the DRB200 Reactor. Preheat to 150 °C.

See the DRB200 User Manual for selecting pre-programmed temperature applications.



4. Remove the caps from two COD Digestion Reagent Vials. (Be sure to use vials for the appropriate range.)

Reactor digestion procedure (continued)

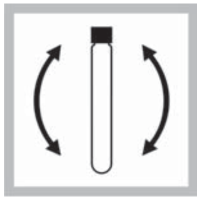
5. Prepared Sample: Hold one vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of sample to the vial.

For the 200–15,000 mg/L vials: Use a TenSette® Pipet to add 0.20 mL of sample to the vial.

6. Blank Preparation: Hold a second vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of deionized water to the vial.

For the 200–15,000 mg/L vials: Use a TenSette Pipet to add 0.20 mL of sample to the vial.

7. Cap the vials tightly. Rinse them with water and wipe with a clean paper towel.



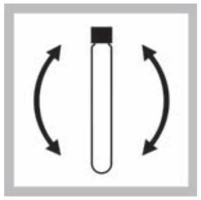
8. Hold the vials by the cap over a sink. Invert gently several times to mix. The sample vials become very hot during mixing.

Insert the vials in the preheated DRB200 Reactor. Close the protective lid.

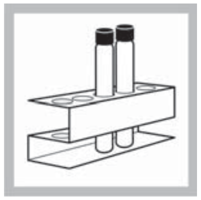
9. Heat the vials for two hours.

10. Turn the reactor off.

Before removing the vials, wait about 20 minutes for the vials to cool to 120 °C or less.



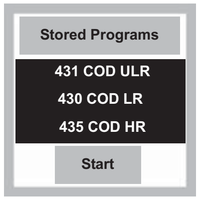
11. Invert each vial several times while still warm.



12. Place the vials into a rack and cool to room temperature.

Proceed to *Colorimetric determination*.

Colorimetric determination



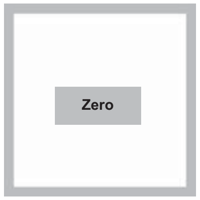
1. Select the test.

Insert an adapter or light shield if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Clean the outside of the vials with a damp towel followed by a dry one.

3. Insert the blank into the 16-mm cell holder.



4. ZERO the instrument.

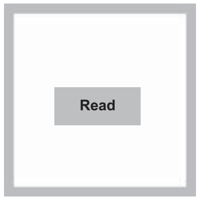
The display will show:

0 mg/L COD

or

0.0 mg/L COD

5. Insert the sample vial into the 16-mm cell holder.



6. READ the results in mg/L COD.

7. If using High Range Plus COD Digestion Reagent Vials, multiply the result by 10.

For most accurate results with samples near 1500 or 15,000 mg/L COD, repeat the analysis with a diluted sample.

Blanks for colorimetric determination

The blank may be used repeatedly for measurements using the same lot of vials. Store the blank in the dark.

1. Monitor decomposition by measuring the absorbance at the appropriate wavelength. Refer to the *Range-specific test wavelengths* table.
2. Zero the instrument in the absorbance mode. Use a vial containing 5 mL of deionized water and measure the absorbance of the blank. Record the value.
3. Prepare a new blank when the absorbance has changed by about 0.01 absorbance units.

Interferences

Chloride is the primary interference when determining COD concentration. Each COD vial contains mercuric sulfate that will eliminate chloride interference up to the level specified in *Column 1* of the *Interfering substances* table. Dilute samples with higher chloride concentrations. Dilute the sample enough to reduce the chloride concentration to the level given in *Column 2*.

Note: *For best results, use the low range and ultra-low range test for samples with high chloride concentrations (approaching maximum concentration) and low COD concentrations.*

If sample dilution will cause the COD concentration to be too low for accurate determination, add 0.50 g of mercuric sulfate ($HgSO_4$) to each COD vial before the sample is added.

The additional mercuric sulfate will raise the maximum chloride concentration allowable to the level given in *Column 3*.

Table 2 Interfering substances

Vial range	**Column 1** (Maximum chloride concentration)	**Column 2** (Suggested chloride concentration for diluted samples)	**Column 3** (Maximum chloride concentration with mercuric sulfate)
Ultra Low Range [1] (0.7–40.0 mg/L)	2000	1000	N/A
Low Range (3–150 mg/L)	2000	1000	8000
High Range (20–1500 mg/L)	2000	1000	4000
High Range Plus (200–15,000 mg/L)	20,000	10,000	40,000

[1] Ultra Low Range is not available on the DR 2700 or the DR/2400.

Sample collection, preservation and storage

- Collect samples in glass bottles.
- Use plastic bottles only if they are known to be free of organic contamination.
- Test biologically active samples as soon as possible.
- Homogenize samples containing solids to assure representative samples.
- Samples treated with sulfuric acid* to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C can be stored up to 28 days.
- Correct the test results for volume additions.

---

* See *Optional reagents and apparatus*.

Accuracy check

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Potassium acid phthalate (KHP), dried overnight at 120 °C
- Deionized water, organic free
- Class A volumetric flasks
- Class A volumetric pipet

0.7 to 40.0 mg/L range

1. Prepare a 30-mg/L COD standard solution as follows:
   - **a.** Dissolve 850 mg of dried KHP in 1000-mL of organic-free deionized water to make a 1000-mg/L standard.
   - **b.** Pipet 3.00 mL of the 1000 mg/L standard into a 100-mL volumetric flask.
   - **c.** Dilute to volume with deionized water and mix well.
2. Use 2 mL of the 30 mg/L COD solution in place of the sample. Follow the *Colorimetric determination* test procedure. The result should be 30 mg/L. Refer to the *Standard adjust* instructions in this procedure to adjust the curve with the reading obtained from the standard.

3 to 150 mg/L range

1. Prepare a 100-mg/L COD standard solution as follows:
   - **a.** Dissolve 85 mg of dried KHP in 1000-mL of organic-free deionized water to make a 1000-mg/L standard.
   - **b.** Pipet 10 mL of the 1000 mg/L standard into a 100-mL volumetric flask.
   - **c.** Dilute to volume with deionized water and mix well.
2. Use 2 mL of the 100-mg/L solution in place of the sample. Follow the *Colorimetric determination* test procedure. The result should be 100 mg/L. Refer to the *Standard adjust* instructions in this procedure to adjust the curve with the reading obtained from the standard

20 to 1500 mg/L range

1. Prepare a 500-mg/L COD standard solution as follows:
   - **a.** Dissolve 425 mg of dried KHP in 1000-mL of organic-free deionized water to make a 500-mg/L standard.
   - **b.** Mix well.
2. Use 2 mL of the 500 mg/L COD solution in place of the sample. Follow the *Colorimetric determination* test procedure. The result should be 500 mg/L. Refer to the *Standard adjust* instructions in this procedure to adjust the curve with the reading obtained from the standard.

   Note: *Alternately, use 2 mL of 300 mg/L, 800 or 1000 mg/L COD standards for accuracy check.*

200 to 15,000 mg/L range

1. Prepare a 10,000-mg/L COD standard solution as follows:
   a. Dissolve 8.500 g of dried KHP in 1000-mL of organic-free deionized water.
2. Use 0.2 mL of the 10,000 mg/L COD solution in place of the sample. Follow the *Colorimetric determination* test procedure. The result should be 10,000 mg/L (after multiplying by 10). Refer to the *Standard adjust* instructions in this procedure to adjust the curve with the reading obtained from the standard.

Standard adjust

1. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	**OPTIONS>MORE>STANDARD ADJUST**
DR 2800	**OPTIONS>MORE>STANDARD ADJUST**
DR 2700	**OPTIONS>MORE>STANDARD ADJUST**
DR/2500	**OPTIONS>STANDARD ADJUST**
DR/2400	**OPTIONS>STANDARD ADJUST**

2. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Alternate reagents

Mercury-free COD2 Reagents can provide a mercury-free testing option for non-reporting purposes. For process control applications, COD2 Reagents will eliminate mercury waste and save on disposal costs. These reagents are fully compatible with test procedures and calibration curves programmed into the spectrophotometer. Determine chloride and ammonia for accurate results.

Important Note: *COD2 reagents are not approved for USEPA reporting purposes. Because COD2 reagents do not contain mercury as a masking agent, they exhibit a positive interference from chloride. Request a copy of the COD Reagent Vial Information Brochure, Lit. No. 1356, for more information about specific applications.*

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
430 (Range, 3–150 mg/L)	DR 5000	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 8000 mg/L COD (High Range Plus)	77–83 mg/L COD	3 mg/L COD
	DR 2800		77–83 mg/L COD	3 mg/L COD
	DR 2700		77–83 mg/L COD	3 mg/L COD
	DR/2500	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 10,000 mg/L COD (High Range Plus)	77.6–82.4 mg/L COD	3 mg/L COD
	DR/2400		77.6–82.4 mg/L COD	3 mg/L COD
431 (Range, 0.5–40.0 mg/L)	DR 5000	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 8000 mg/L COD (High Range Plus)	28.8–31.2 mg/L COD	0.5 mg/L COD
	DR 2800		28.8–31.2 mg/L COD	0.5 mg/L COD
431 (Range, 0.7–40.0 mg/L)	DR/2500	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 10,000 mg/L COD (High Range Plus)	29.0–31.0 mg/L COD	0.7 mg/L COD
435 (Range, 20–1500 mg/L)	DR 5000	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 8000 mg/L COD (High Range Plus)	785–815 mg/L COD	23 mg/L COD
	DR 2800		785–815 mg/L COD	23 mg/L COD
	DR 2700		785–815 mg/L COD	23 mg/L COD
	DR/2500	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 10,000 mg/L COD (High Range Plus)	778–822 mg/L COD	20 mg/L COD
	DR/2400		778–822 mg/L COD	20 mg/L COD
435 (Range, 200–15,000 mg/L)	DR 5000	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range);8000 mg/L COD (High Range Plus)	7850–8150 mg/L COD	230 mg/L COD
	DR 2800		7850–8150 mg/L COD	230 mg/L COD
	DR 2700		7850–8150 mg/L COD	230 mg/L COD
	DR/2500	30 mg/L COD (Ultra Low Range); 80 mg/L COD (Low Range); 800 mg/L COD (High Range); 10,000 mg/L COD (High Range Plus)	9780–10,220 mg/L COD	200 mg/L COD
	DR/2400		9780–10,220 mg/L COD	200 mg/L COD

Summary of method

The results in mg/L COD are defined as the milligrams of O_2 consumed per liter of sample under the conditions of this procedure. The sample is heated for two hours with sulfuric acid and a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}).

When the 0.7–40.0 or the 3–150 mg/L colorimetric method is used, the amount of Cr^{6+} remaining is determined. When the 20–1500 mg/L or 200–15,000 mg/L colorimetric method is used, the amount of Cr^{3+} produced is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences.

Test results are measured at the wavelengths specified in the *Range-specific test wavelengths* table.

Table 3 Range-specific test wavelengths

Range in mg/L COD	Wavelength
0.7 to 40.0 mg/L[1]	350 nm
3 to 150 mg/L	420 nm
20 to 1500	620 nm
2000 to 15,000 mg/L	620 nm

[1] Not available on the DR/2400 or the DR 2700

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Select the appropriate COD Digestion Reagent Vial:			
Ultra Low Range, 0.7 to 40 mg/L COD	1–2 vials	25/pkg	2415825
Low Range, 3 to 150 mg/L COD	1–2 vials	25/pkg	2125825
High Range, 20 to 1500 mg/L COD	1–2 vials	25/pkg	2125925
High Range Plus, 200 to 15,000 mg/L COD	1–2 vials	25/pkg	2415925
Water, deionized	varies	4 L	27256

Alternate reagents[1]

Description	Quantity/Test	Unit	Catalog number
Select the appropriate COD Digestion Reagent Vial:			
COD2, Low Range, 0 to 150 mg/L COD	1–2 vials	25/pkg	2565025
COD2, High Range, 0 to 1500 mg/L COD	1–2 vials	25/pkg	2565125
COD2, High Range, 0 to 1500 mg/L COD	1–2 vials	150/pkg	2565115
COD2, High Range Plus, 0 to 15,000 mg/L COD	1–2 vials	25/pkg	2834325
COD Digestion Reagent Vials, 3 to 150 mg/L COD	—	150/pkg	2125815
COD Digestion Reagent Vials, 200 to 1500 mg/L COD	—	150/pkg	2125915

[1] These reagents are not approved for USEPA reporting purposes. Request a copy of the COD Reagent Vial Information Brochure, Lit. No. 1356, for more information about specific applications.

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Blender, 2-speed, 120 VAC	1	each	2616100
Blender, 2-speed, 240 VAC	1	each	2616102
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Pipet Filler, safety bulb	1	each	1465100
Pipet, Volumetric, Class A, 2.00 mL	1	each	1451536

COD Laboratory

Samples: Blanks (deionized water)
Screened raw influent
Oxidation ditch effluent – settle supernatant
Plant effluent
COD Standard

Sample	**Vial No.**	**COD, mg/L**
Blank – High Range	______	__________
Standard - ______	______	__________
Screened Influent	______	__________
Oxidation Ditch Effluent	______	__________
Plant Effluent	______	__________
Duplicate	______	__________
Blank – Low Range	______	__________

Section 11
Turbidity

Turbidity

Laboratory Workshop

TENNESSEE DEPARTMENT OF ENVIRONMENT AND CONSERVATION

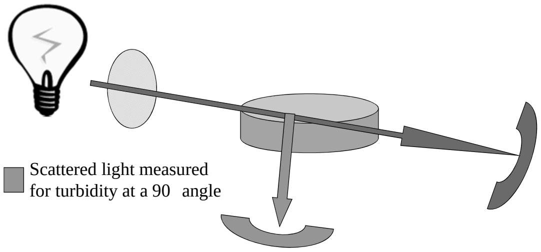
Turbidity

- A measure of the clarity of water
- It is an expression of the optical property that causes light to be scatter and absorbed in water
- It is caused by particulate, such as silt, clay, organic matter, algae and other microorganisms
- Amount of light absorbed is proportional to the concentration of particulate in the sample

Turbidity

- Caused by suspended and colloidal matter in water
- It is expression of light that is scattered or absorbed through a sample
- Does not indicate the number or size of particles in a sample
- General indicator of overall effluent water quality and a good process control test for operator

Turbidimeters



▨ Scattered light measured for turbidity at a 90 angle

▨ Light source from tungsten lamp passing through three precisely aligned lenses, the light is focused in a narrow, collimated beam

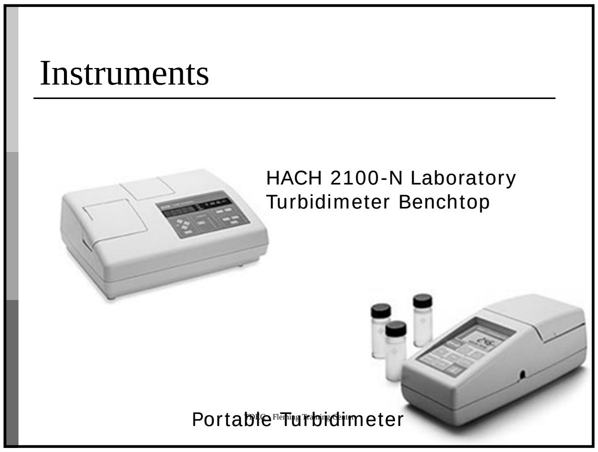
Importance

- Supports growth of microorganisms
- Reduces effectiveness of chlorination
- Interferes with chemical and microbiological analysis
- Is unacceptable for aesthetic reasons
- Is related to coagulation and filtration
- Is unacceptable for most industrial water

Measuring

- Use an instrument for measuring and comparing turbidity of liquids
- Nephelometers are instruments which measure turbidity by comparing the amount of light in a sample to the amount of light scattered by a standard
- The amount of scattered light is measured and converted to units of turbidity or NTU's (Nephelometric Turbidity Units)

Instruments



HACH 2100-N Laboratory Turbidimeter Benchtop

Portable Turbidimeter

Measuring Notes

- Always cap the sample cell to prevent spillage into instrument
- Close the sample compartment lid during measurement
- Do not leave sample cell in the cell compartment for extended periods of time
- Leave the instrument on 24 hours a day if instrument is used regularly

Measuring Notes

- Always use clean, scratch free sample cells and caps
- Always use silicone oil
- Measuring samples immediately to prevent changes in sample characteristics
- Remove air bubbles in sample cells
- Discard sample cells with scratches

Calibrations

- Use Gelex Secondary Turbidity Standards for periodic checks
- Primary Stable Cal Standards
  - Formazin Solution Primary Standards and Procedure for making solutions
- Record keeping requirements and recommendations for operators
- Calibrate at least quarterly

47000-44

HACH® 2100N LABORATORY TURBIDIMETER QUICK REFERENCE GUIDE

NEPHELOMETRIC MEASUREMENT PROCEDURE

1. Collect a representative sample in a clean container. Fill the sample cell to the line (approximately 30 mL). Take care to handle the sample cell by the top. Cap the sample cell. (*Note: Instrument warm-up stabilization time with Ratio on is 30 minutes and with Ratio off is 60 minutes. Typical application is to leave the instrument on 24 hours a day.)*

2. Hold the sample cell by the cap, and wipe to remove water spots and finger prints.

3. Apply a thin bead of silicone oil from the top to the bottom of the cell—just enough to coat the cell with a thin layer of oil. Using the oiling cloth provided, spread the oil uniformly. Then, wipe off the excess. The cell should appear nearly dry with little or no visible oil. (*Note: See Section 2.3.2 Applying Silicone Oil in the instrument manual.)*

4. Place the sample cell in the instrument cell compartment, and close the cell cover. (*Note: For immediate update of the display, press* **ENTER.**)

5. If necessary, insert the EPA filter. Select manual or automatic ranging by pressing the **RANGE** key.

6. Select the appropriate **SIGNAL AVERAGING** setting (on or off) by pressing the **SIGNAL AVG** key.

7. Select the appropriate **RATIO** setting (on or off) by pressing the **RATIO** key. (*Note: Values >40 NTU require Ratio on*.)

8. Select the appropriate measurement unit (NTU, EBC or NEPH) by pressing the **UNITS/EXIT** key.

9. Read and record the results.

CALIBRATION

Preparing Recommended Formazin Dilutions

Hach Company recommends use of 20-, 200-, 1000- and 4000-NTU Formazin standards for calibration of the Model 2100N Turbidimeter. Prepare all Formazin dilutions immediately before calibration, and discard the dilutions after use. While 4000-NTU stock solutions are stable for up to one year, diluted solutions deteriorate more rapidly. Prepare dilutions of 20, 200 and 1000 NTUs according to the directions in *Table 2 (Formazin Standard Preparation)* in *Section 3* of the Instrument Manual. The dilution water also is used to make an initial blank measurement (refer to *Section 3.2 Calibration* in the Instrument Manual).

NOTE

The calibration is based on a first order linear equation consisting of up to three independent variables. Unpredictable results may occur if standards other than the recommended calibration points are used. The factory-suggested calibration points are those determined by Hach Company chemists and engineers to provide the best calibration accuracy. Use of standards other than those specified may result in less accurate calibrations.

Calibrating with Formazin Standards

The electronic and optical design of the 2100N Turbidimeter provides long-term stability and minimizes the need for frequent calibration. The three-detector ratioing optical system compensates for electronic and optical system variations between calibrations. When data is used for USEPA reporting, recalibrate at least every 90 days, or as stipulated by the regulating authority. Refer to *Section 3.2 Calibration* in the Instrument Manual.

1. Fill a clean sample cell to the line (≅ 30 mL) with dilution water. Wipe the cell clean and apply a thin film of silicone oil.

2. Place the sample cell into the cell holder, and *close the cell cover*.

3. Press the **CAL** key. The S0 annunciator lights. The NTU value of the dilution water used in the previous calibration is displayed.

4. Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. This result is stored and used to compensate for the turbidity of the dilution water.

5. The instrument automatically increments to the next standard, displays the expected NTU value (e.g., 20.00 NTU), and the S1 annunciator lights. Remove the sample cell from the cell holder.

6. Fill a clean sample cell to the line with well-mixed, 20-NTU Formazin standard. Wipe the sample cell clean, and apply a thin film of silicone oil on its surface. Place it into the cell holder, and *close the cell cover*.

7. Press the **ENTER** key. The display counts down from 60 to 0, and makes a measurement. The instrument automatically increments to the next standard, the display shows 200.0 NTU, and the S2 annunciator lights. Remove the sample cell from the instrument.

8. Fill a clean sample cell to the line with well-mixed, 200-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it into the cell holder, and *close the cell cover*. Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. The instrument automatically increments to the next standard, the display shows 1000 NTU, and the S3 annunciator lights. Remove the sample cell from the instrument.

9. Fill a clean sample cell to the line with well-mixed, 1000-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it in the cell holder and *close the cell cover*. Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. The display automatically increments to the next standard, the display shows 4000 NTU, and the S4 annunciator lights. Remove the sample cell from the instrument.

10. Fill a clean sample cell to the line with well-mixed, 4000-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it in the cell holder and *close the cell cover*. Press the **ENTER** key. The instrument counts down from 60 to 0, and then makes a measurement. The display automatically increments back to the dilution water standard. The S0 annunciator lights, and the previously measured value of the dilution water is displayed.

11. Press the **CAL** key. The instrument makes calculations based on the new calibration data, stores the new calibration and returns the instrument to the measurement mode.

Reviewing the Calibration Sequence

Press the **CAL** key and then use the **UP ARROW** key to scroll through the standards to review calibration data currently in effect. If the instrument is connected to a printer, pressing the **PRINT** key prints all of the calibration data in effect. Press the **UNITS/EXIT** key to return to the operating mode without altering the current calibration data.

Using Gelex® Secondary Turbidity Standards

Periodically, as experience or regulating authorities indicate, verify the instrument calibration using Gelex Secondary Standards. If the reading in the range of use is not within 5% of the standard's assigned value, recalibrate using Formazin primary standards (refer to *Section 3.2.5 Using Gelex Secondary Turbidity Standards* in the Instrument Manual).

1. Calibrate the instrument with Formazin (refer to *Section 3.2 Calibration* in the Instrument Manual).

2. Verify that the instrument is set for the NTU mode, Ratio on and Automatic Ranging.

3. Thoroughly clean the outside of the Gelex vials, and apply a thin coating of silicone oil.

4. Place the lowest NTU Gelex Standard in the sample compartment with the triangle on the vial aligned with the index mark on the instrument sample compartment. Close the sample cell cover.

5. Press the **ENTER** key. Record the value displayed. Remove the standard from the instrument, and mark this value on the vial with a water soluble marker.

6. Repeat steps 3 through 5 for the other Gelex standards.

NOTE

Reassign new values to the Gelex standards each time the instrument is calibrated with Formazin.

ERROR CODES

Error codes may result from instrument malfunction or operator error. **Errxx** error codes are cleared from the display by pressing the **ENTER** key. The meter continues operating in the error condition; a calibration in progress can be continued. Any calibration being calculated (at the time the message appears) is discarded; the old calibration is retained. *Table 1* lists the error codes displayed for specific conditions.

Table 1. Error Codes

Code	Probable Cause	Corrective Action
Err01	Dilution water calculated to be >0.5 NTU	Start calibration over with higher quality dilution water, or filter the water with a membrane filter before use.
Err02	Two calibration standards have the same value, or their difference is less than 60.0 NTU. Standard 1 is too low (<10 NTU)	Recheck preparation of standards and repeat calibration.
Err03	Low light error	Reinsert sample. Check that lamp is on. Dilution may be necessary.
Err04	Memory malfunction	Switch instrument off and back on with I/O. Call Hach Service.
Err05	A/D over-range	Contact Hach Service.
Err06	A/D under-range	Contact Hach Service.
Err07	Light leak	Contact Hach Service.
Err08	Bad lamp circuit	Contact Hach Service.
Err09	Printer timeout error	Check that external printer is properly connected. Check that external printer is selected (on-line).
Err10	System voltage out of range	Switch instrument off and back on with I/O. Call Hach Service.
Err11	System loop test error	Switch instrument off and back on with I/O. Call Hach Service.

Diagnostic Functions

The diagnostic mode accesses system function information that is useful primarily when the instrument function is in doubt. Hach service technicians use the information for precise troubleshooting, speeding repairs, and avoiding unnecessary service returns.

Access diagnostic information by pressing and holding the **RIGHT ARROW** key for 3 seconds. Use the **ARROW** keys to edit the display to read the diagnostic code number of interest. Press the **ENTER** key to display the diagnostic value. More information may be obtained by purchasing the instrument service manual, or contacting the service center nearest you.

Diagnostic Codes

Code	Display	Description
00	bP on/bP of	Keyboard Beeper On/Off
01	FS Pr/SL Pr	Fast/Slow Print Device
21	Pr In	Printer Test
22	*	Display Test
23	*	Keyboard Test
24	*	Memory Test

Refer to *Table 6 Diagnostic Codes* in *Section 8 Troubleshooting* of the instrument manual for a list of diagnostic codes.

HACH COMPANY
WORLD HEADQUARTERS
P.O. BOX 389
Loveland, Colorado 80539
Telephone: (970) 669-3050
FAX: (970) 669-2932

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Section 12
Jar Testing

JAR TESTING

Wastewater Lab

Solids Removal Using Chemicals

- Physical-chemical treatment
- Three step process, must occur in proper sequence
  - Coagulation Phase: Chemicals are added to the wastewater and rapidly mixed forming "pinpoint floc"
  - Flocculation Phase: Gentle mixing to produce larger, denser floc particles that settle rapidly
  - Liquid/solids separation is almost always conventional sedimentation by gravity settling although air flotation is used occasionally

Most Important Guidelines

- Provide enough energy to completely mix chemicals
- Control intensity of mixing during flocculation
- Control chemical(s) dose

Principles of Coagulation

- Coagulation involves both chemical (destabilization) and physical (mixing) processes.
- Coagulation produces solid particles that form strong enough to withstand shearing

Principles of Coagulation

- Van Der Waals Force: attractive force existing between particles that allows coagulation to occur
- Zeta Potential: Measurement in millivolts of particle strength surrounding solids. The more negative the number, the stronger particle charge and repelling forces between particles

Principles of Coagulation

- To achieve coagulation the electrostatic charges of particles must be modified to reduce Zeta Potential
- Add chemicals that have charge opposite of the suspended solids
- Wastewater tends to have a negative ionic charge and pH of 6.5

Flocculation Process

- Most precise application of flocculation is in the preparation of secondary effluents for tertiary filtration use to "polish" the effluent.
- Simple coagulation not sufficient to enlarge suspended solids particles enough to meet pre-filtration conditioning requirements.
- If suspended solids less than 15 mg/L leaving the secondary treatment process polymers use

Flocculation Process

- Selection of polymers depends on pH, conductivity, type and concentration of suspended solids, particle size ranges, type and amount of coagulant(s) added, and next stage in treatment process
- Most important to have sufficient paddle speeds during flocculation to keep floc from settling and not shearing (adjustable speed drives)

Chemicals Used to Improve Settling

- Aluminum Sulfate (Dry or Liquid)
- Ferric Chloride
- Lime
- Polymers

Aluminum Sulfate

- Very corrosive when mixed with water
- Supports bacterial growth and/or cause sludge deposits in feed lines
- Reduces alkalinity in water being treated during the coagulation process
- Hydrated lime, soda ash, or caustic soda may be required to adjust alkalinity for coagulation process to occur
- A 1% solution will have a pH of 3.5
- Alkalinity adjustment: 1 mg/L of chemical for alkalinity adjustment when using Alum

Ferric Chloride

- Very Corrosive
- Will leave stains
- Temperature rises as chemical dissolves
- Crystallizes in temperatures below -1°C
- Positive displacement pumps should be used for accuracy
- Will lower pH

Lime

- Use to coagulate solids or adjust pH to improve coagulation
- Very irritating to skin, eyes, and mucous membranes
- High heat is generated when water is added to chemical
- Quicklime less expensive but slaking required

Polymers

- High molecular weight organic compounds
- Natural or synthetic origin
- Wide range available
- May be applied alone or in combination with other chemicals
- Extensive laboratory tests
- Anionic, Nonionic, and Cationic Polymers

Selection of Chemicals and Dosages

- Three Step Process
  - Preliminary Screening
  - Dosage Testing
  - Full-Scale Trial
- Tested water treated must be freshly drawn samples from actual flow stream
- System performance optimization: regular testing of water upstream of chemical application

Jar Testing

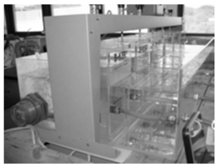
- Basic guidelines:
  - Jar test volume 2 Liters
  - working chemical strengths should be 0.1% (1,000 mg/L)
  - Rapid mix at 140-160 RPM for 10-15 seconds for polymers: 3-5 minutes for aluminum and/or iron metal salts (Ferric Chloride)
  - Slow mix 15-20 RPM for 1-2 minutes for polymers; slow mix not used for aluminum and/or iron metal salts

Jar Testing

- Ranges for times represent normal operating conditions.
- Actual times based on composition of flow stream examined (detention and force gradients)

Water Bath

- Jars set in a rectangular tank with raw water circulating around them
- This is necessary for water that is not room temperature



Water Bath

- Determination for use of water bath:
  - Take a raw sample
    - Run jar test immediately
  - Take another sample
    - Let warm 5-10 degrees
    - Run same test and take note in difference

Solution Preparation

- Accuracy is critical - Small errors are compounded by large dilution
- Dilute solutions of 1g/L or less of coagulants or polymers should be prepared daily

Solution Prep - Dry Products

- Dissolve 1 gram of a chemical that is 100% in 1000 ml of DI water
  - This is a 0.1% solution by weight or 1,000 mg/L
- In a 1 liter test beaker, 1 ml of the above equals 1 mg/L

- If the chemical is not 100%, then divide by the percent of the chemical available

Solution Prep - Dry Products

- Useful dilution for alum, iron salts, carbons and alkalis

Dry Alum

- Use a 1-L volumetric flask
- Dissolve 10 grams in 600 mL DI water
- Fill to the mark and mix

- Solution contains 10,000 mg/L
- Therefore, 1mL of the stock solution added to a 2-L jar will equal a 5 mg/L alum dose

Solution Prep - Liquid Products

- To make a 10 g/L solution (1%) of a liquid product, divide 10 gm by specific gravity
  - This is the chemical to dissolve in 1000 ml DI water
  - In a 1 liter test beaker, 1 ml of the above equals 10 mg/L of liquid product
- For solutions like PACl

Remember, Jar Test beakers are usually 2L, therefore double dose

Solution Prep - Liquid Products

- For liquids sold on a dry basis, correct for concentration as follows
  - 10 gm/(sp.grav. x conc.)
  - In a 1 liter test beaker, 1 ml of the above equals 10 mg/L of liquid product
- For solutions like alum, ferric

Again, remember Jar Test beakers are usually 2L, therefore double dose

Solution Prep - Liquid Products

- Example:
  - Liquid aluminum sulfate is typically sold on a dry basis.
  - 48.5% $Al_2(SO_4)_3 \bullet 14\ H_2O$ and specific gravity of 1.335
  - 10g/L/(1.335 x 0.485) = 15.4 ml liquid alum for a 10 gram/liter dry basis solution.
  - 1 ml of this solution = 10 mg/L dry alum

Solution Prep - Liquid Products

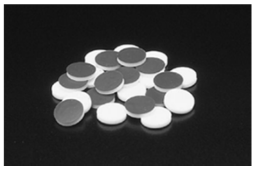
- Micropipets dispense neat product
- Specific gravity must be accounted for (liquid chemicals weigh more than water, so 1 ml of product weights more than 1 gram)
  - $\frac{\text{mg/L}}{\text{sp. grav.}}$ = µL product / liter of test sol'n

Solution Prep - Liquid Products

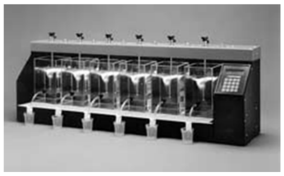
- For dose on a dry active product basis, divide by concentration:
  - $\frac{\text{mg/L}}{\text{(sp.grav. x conc.)}}$ = µL product/L of test sol'n

Micro-pipeting

- Try to place dose on an object that can perch on the rim of the jar until you are ready to add all at the same time
  - Septas from TOC vials
  - Powder pillows



Jar Testing



- Must know volume of test jars and speed rates of stirrers prior to test
- Don't use laboratory grade chemicals
  - Use what you feed in plant

References

- Cal State Sacramento Advanced Waste Treatment Edition 3 Chapter 4. Solids Removal from Secondary Effluents
- Practical Manual of Wastewater Chemistry by Barbara A. Hauser, 1996, ISBN 1-57504-012-3, Ann Arbor Press, Inc.
- Water Treatment: Troubleshooting and Problem Solving, 1996, by Glenn M. Tillman ISBN 1-57504-001-8
- CRC Press LLC

Section 13
Total Chlorine

Wastewater Laboratory

CHLORINE DISINFECTION

Chlorine Uses in WWTP

- Disinfection
- Reduce BOD
- Odor control
- Improve scum and grease removal
- Control activated sludge bulking
- Foam control
- Filter fly control

Disinfection

- Disinfection is the process that destroys pathogens.
- This is usually through the addition of chlorine.
- Other methods:
  - Heat
  - Bromine
  - Iodine
  - Ozone
  - UV

Disinfection

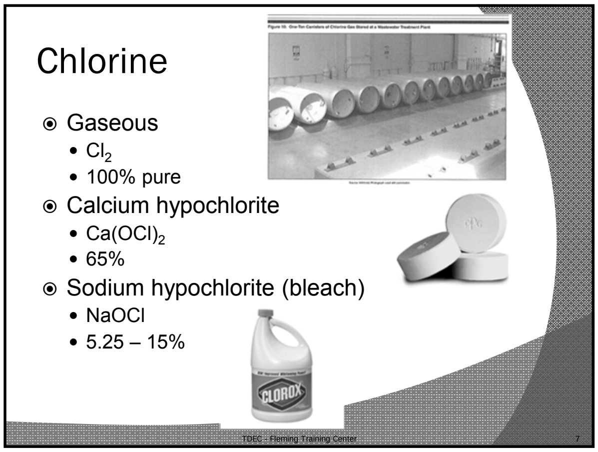
- The most important purpose of chlorination of wastewater is disinfection of the plant effluent.
- It minimizes the potential health hazard to humans from waterborne diseases.
- The amount of chlorine necessary to obtain a satisfactory reduction of bacteria will vary greatly with each plant effluent.
- Do not overchlorinate!!!
- Remember the amount of chlorine required will decrease as the quality of your plant effluent improves.

Odor Control

- Anaerobic conditions will produce hydrogen sulfide with its characteristic rotten egg smell.
- Chlorine can break down hydrogen sulfide as well as other odor producing bacteria.

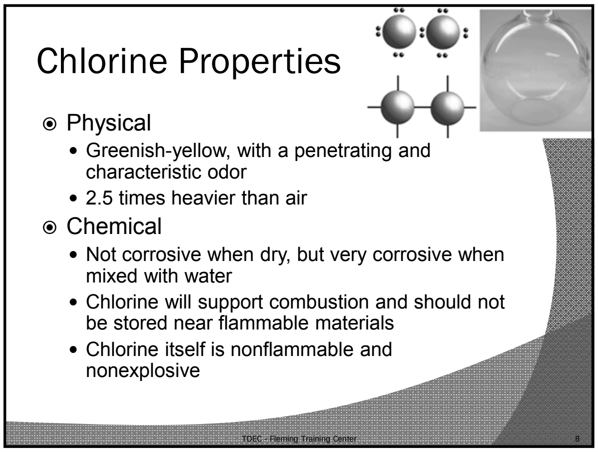
Control of Activated Sludge Bulking

- When sludge bulking has been traced to filamentous organisms and the situation doesn't improve by adjusting F:M ratio and nutrient levels, then chlorination may help.
- Chlorination should only be continued until the filamentous population has decreased and a normal bacteria population has established itself.
- Do not overchlorinate, as you could kill the whole process
- The chlorine is added to the return sludge line



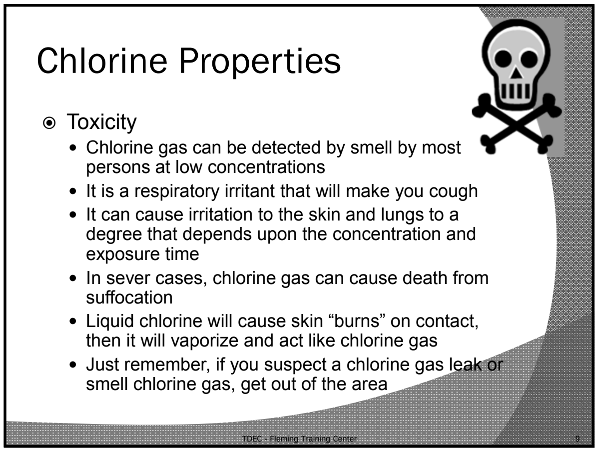
Chlorine

- Gaseous
  - Cl_2
  - 100% pure
- Calcium hypochlorite
  - $Ca(OCl)_2$
  - 65%
- Sodium hypochlorite (bleach)
  - NaOCl
  - 5.25 – 15%



Chlorine Properties

- Physical
  - Greenish-yellow, with a penetrating and characteristic odor
  - 2.5 times heavier than air
- Chemical
  - Not corrosive when dry, but very corrosive when mixed with water
  - Chlorine will support combustion and should not be stored near flammable materials
  - Chlorine itself is nonflammable and nonexplosive



Chlorine Properties

- Toxicity
  - Chlorine gas can be detected by smell by most persons at low concentrations
  - It is a respiratory irritant that will make you cough
  - It can cause irritation to the skin and lungs to a degree that depends upon the concentration and exposure time
  - In sever cases, chlorine gas can cause death from suffocation
  - Liquid chlorine will cause skin “burns” on contact, then it will vaporize and act like chlorine gas
  - Just remember, if you suspect a chlorine gas leak or smell chlorine gas, get out of the area

Storage of Chlorine

- Do not store in the same room as the dechlorinator (sulfur dioxide, sodium bisulfite, sodium metabisulfite, etc.)
- Exhaust fan intake and exhaust for chlorine storage room located at floor level
- Switches for fans and lights outside cylinder room
- Ammonium hydroxide on hand to test for leaks
- If you do have a leak: 2 in 2 out - required for emergencies; DO NOT BE A HERO

Affects on pH

- Hypochlorination causes pH to ↑

$$2NaOCl + 2H_2O \rightarrow 2\mathbf{NaOH} + HOCl + OCl^- + H^+$$

$$Ca(OCl)_2 + H_2O \rightarrow HOCl + \mathbf{Ca(OH)_2}$$

- Gas chlorination causes pH to ↓

$$Cl_2 + H_2O \rightarrow HOCl + \mathbf{HCl}$$

Chlorine Demand

- The difference between the chlorine added to the water and the amount of residual chlorine remaining after a given time.

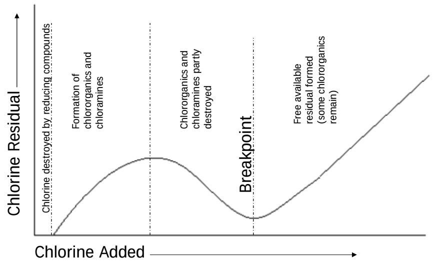
Demand = Chlorine Dose – Chlorine Residual

Dose = Chlorine Demand + Chlorine Residual

- Wastewater is not pure.
- The reaction of chlorine with impurities interferes with the formation of a free chlorine residual.

Fe^{++}	Inorganic Compounds
Mn^{++}	Nitrogen Compounds
Microorganisms	

Chlorine Breakpoint



Chlorine Residual Compliance

- Sample NPDES Permit states on page 31 of 32:
  - "This permit contains a residual chlorine limit of 0.02 mg/L based on the instream protection value of 0.019 mg/L for fish and aquatic life. The current limit of detection for residual chlorine is 0.05 mg/L. The Permittee shall obtain the equipment that is necessary to test for residual chlorine down to the detection level. Detection of chlorine in any test will constitute a violation of the permit."

Chlorine Testing Methods

- ***Amperometric Titration (back)***
- ***DPD Colorimetric***
- Starch end-point
- DPD Titrimetric
- ***Ion Specific Electrode***

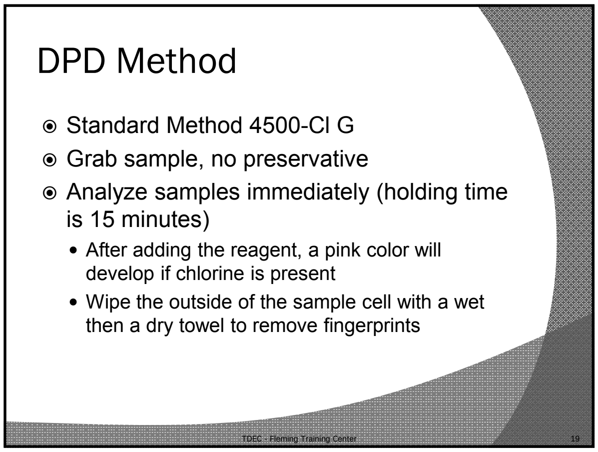
*****These methods will yield results at the 0.05 mg/L detection level***

Total vs. Free Chlorine

- Free chlorine refers to both hypochlorous acid (HOCl) and the hypochlorite (OCl^-) ion or bleach, and is commonly added to water systems for disinfection.
- When ammonia or organic nitrogen is also present, chloramines known as monochloramine, dichloramine, and trichloramine will quickly form.
- Chloramines are also known as combined chlorine.
- Total chlorine is the sum of free chlorine and combined chlorine.

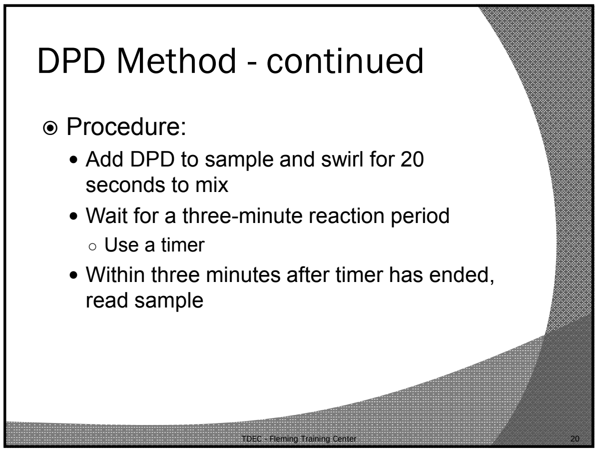
Total vs. Free Chlorine

- The level of total chlorine will always be higher than or equal to the level of free chlorine.
- Free chlorine is typically measured in drinking water disinfection systems using chlorine gas or sodium hypochlorite to find whether the water system contains enough disinfectant.
  - Typical levels of free chlorine in drinking water are 0.2 - 2.0 mg/L Cl_2, though levels can be as high as 5.0 mg/L.
- Total chlorine is typically measured to determine the total chlorine content of treated wastewater, often for discharge purposes.



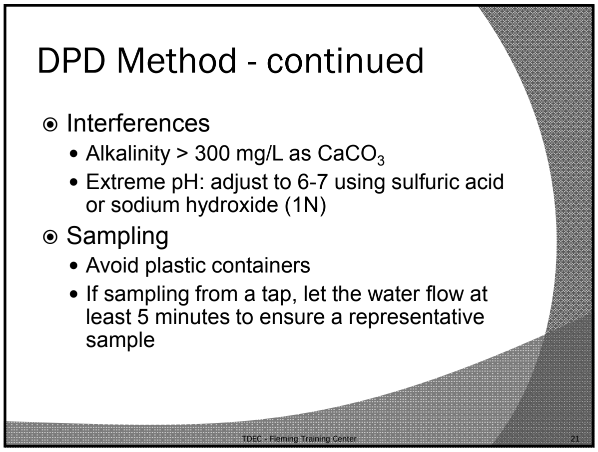
DPD Method

- Standard Method 4500-Cl G
- Grab sample, no preservative
- Analyze samples immediately (holding time is 15 minutes)
  - After adding the reagent, a pink color will develop if chlorine is present
  - Wipe the outside of the sample cell with a wet then a dry towel to remove fingerprints



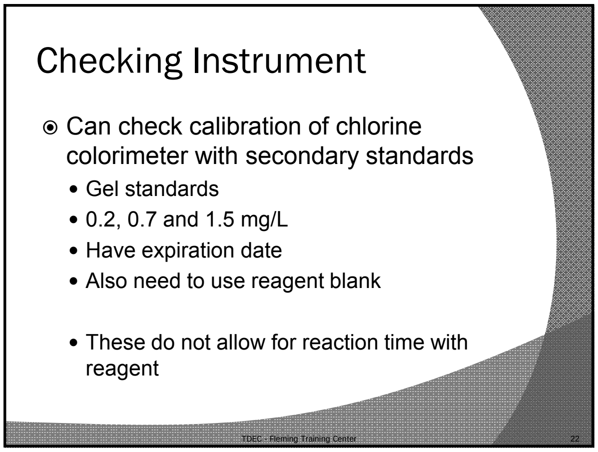
DPD Method - continued

- Procedure:
  - Add DPD to sample and swirl for 20 seconds to mix
  - Wait for a three-minute reaction period
    - Use a timer
  - Within three minutes after timer has ended, read sample



DPD Method - continued

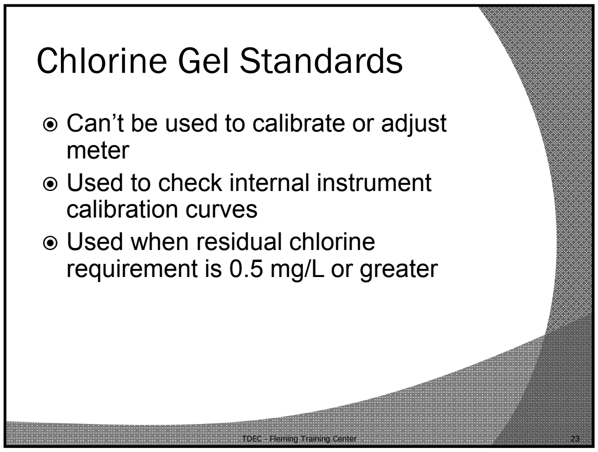
- Interferences
  - Alkalinity > 300 mg/L as $CaCO_3$
  - Extreme pH: adjust to 6-7 using sulfuric acid or sodium hydroxide (1N)
- Sampling
  - Avoid plastic containers
  - If sampling from a tap, let the water flow at least 5 minutes to ensure a representative sample



Checking Instrument

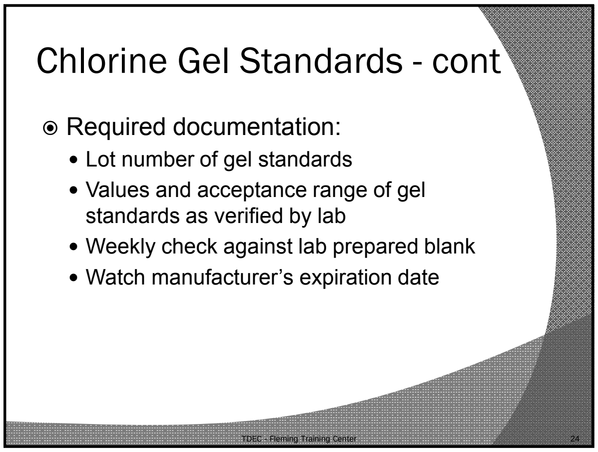
- Can check calibration of chlorine colorimeter with secondary standards
  - Gel standards
  - 0.2, 0.7 and 1.5 mg/L
  - Have expiration date
  - Also need to use reagent blank

  - These do not allow for reaction time with reagent



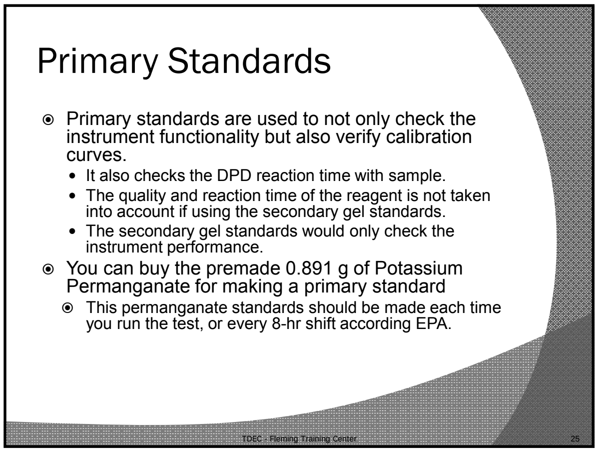
Chlorine Gel Standards

- Can't be used to calibrate or adjust meter
- Used to check internal instrument calibration curves
- Used when residual chlorine requirement is 0.5 mg/L or greater



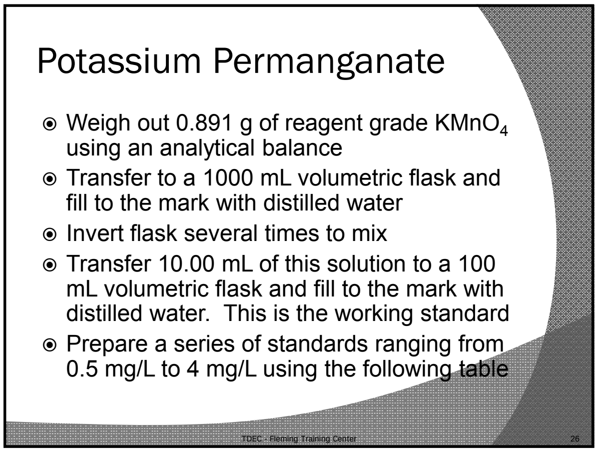
Chlorine Gel Standards - cont

- Required documentation:
  - Lot number of gel standards
  - Values and acceptance range of gel standards as verified by lab
  - Weekly check against lab prepared blank
  - Watch manufacturer's expiration date



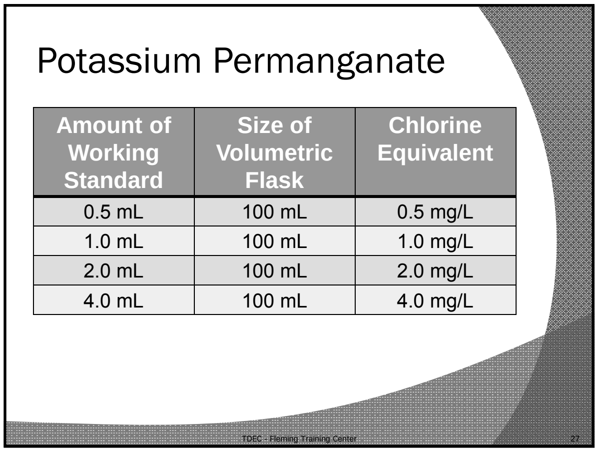
Primary Standards

- Primary standards are used to not only check the instrument functionality but also verify calibration curves.
  - It also checks the DPD reaction time with sample.
  - The quality and reaction time of the reagent is not taken into account if using the secondary gel standards.
  - The secondary gel standards would only check the instrument performance.
- You can buy the premade 0.891 g of Potassium Permanganate for making a primary standard
  - This permanganate standards should be made each time you run the test, or every 8-hr shift according EPA.



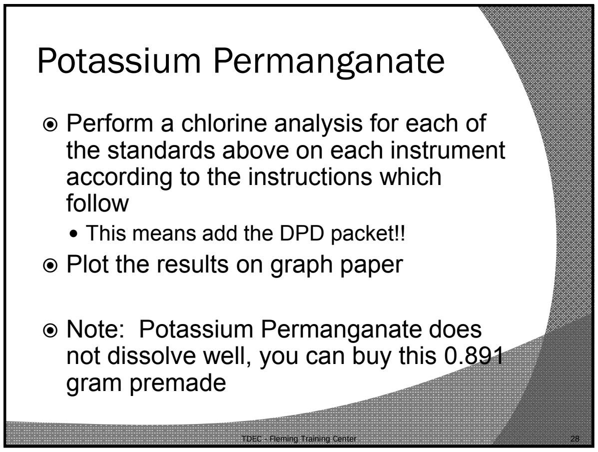
Potassium Permanganate

- Weigh out 0.891 g of reagent grade $KMnO_4$ using an analytical balance
- Transfer to a 1000 mL volumetric flask and fill to the mark with distilled water
- Invert flask several times to mix
- Transfer 10.00 mL of this solution to a 100 mL volumetric flask and fill to the mark with distilled water. This is the working standard
- Prepare a series of standards ranging from 0.5 mg/L to 4 mg/L using the following table



Potassium Permanganate

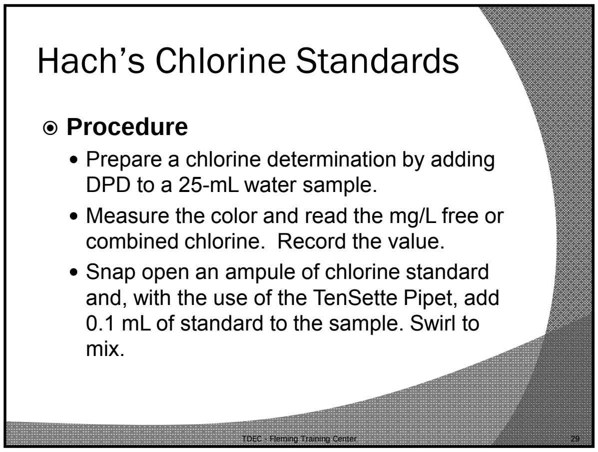
Amount of Working Standard	Size of Volumetric Flask	Chlorine Equivalent
0.5 mL	100 mL	0.5 mg/L
1.0 mL	100 mL	1.0 mg/L
2.0 mL	100 mL	2.0 mg/L
4.0 mL	100 mL	4.0 mg/L



Potassium Permanganate

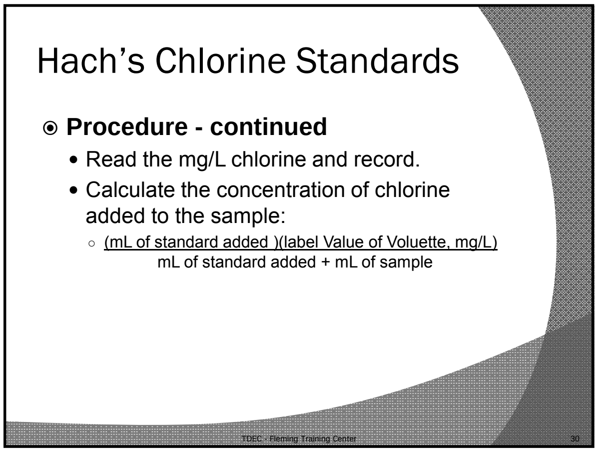
- Perform a chlorine analysis for each of the standards above on each instrument according to the instructions which follow
  - This means add the DPD packet!!
- Plot the results on graph paper

- Note: Potassium Permanganate does not dissolve well, you can buy this 0.891 gram premade



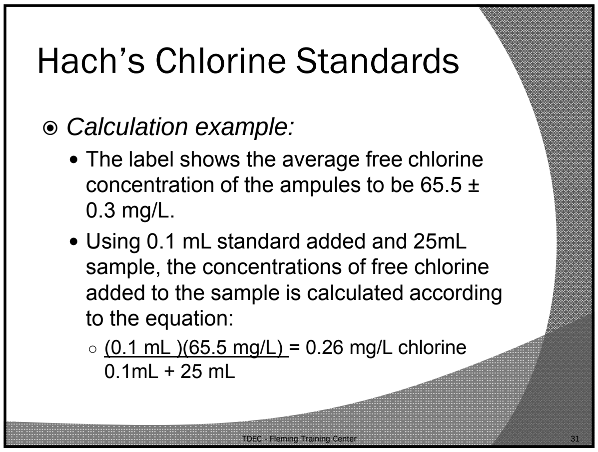
Hach's Chlorine Standards

- **Procedure**
  - Prepare a chlorine determination by adding DPD to a 25-mL water sample.
  - Measure the color and read the mg/L free or combined chlorine. Record the value.
  - Snap open an ampule of chlorine standard and, with the use of the TenSette Pipet, add 0.1 mL of standard to the sample. Swirl to mix.



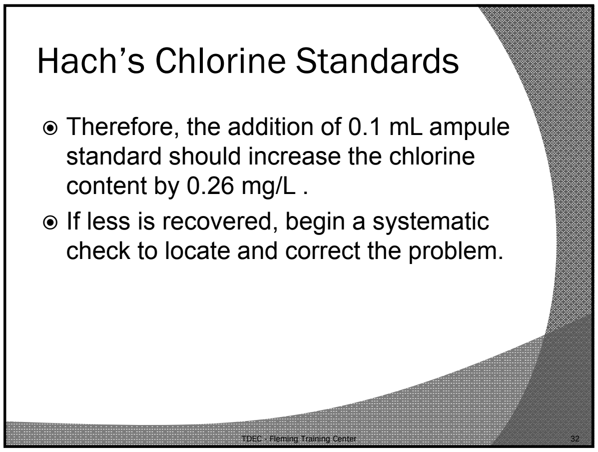
Hach's Chlorine Standards

- **Procedure - continued**
  - Read the mg/L chlorine and record.
  - Calculate the concentration of chlorine added to the sample:
    - $\frac{\text{(mL of standard added)(label Value of Voluette, mg/L)}}{\text{mL of standard added + mL of sample}}$



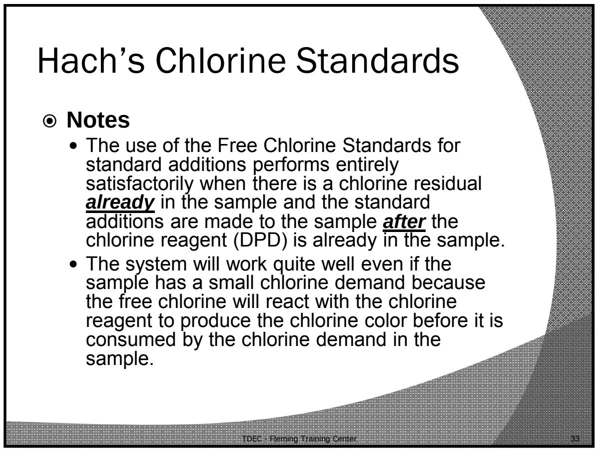
Hach's Chlorine Standards

- *Calculation example:*
  - The label shows the average free chlorine concentration of the ampules to be 65.5 ± 0.3 mg/L.
  - Using 0.1 mL standard added and 25mL sample, the concentrations of free chlorine added to the sample is calculated according to the equation:
    - $\frac{(0.1\text{ mL})(65.5\text{ mg/L})}{0.1\text{mL} + 25\text{ mL}} = 0.26$ mg/L chlorine



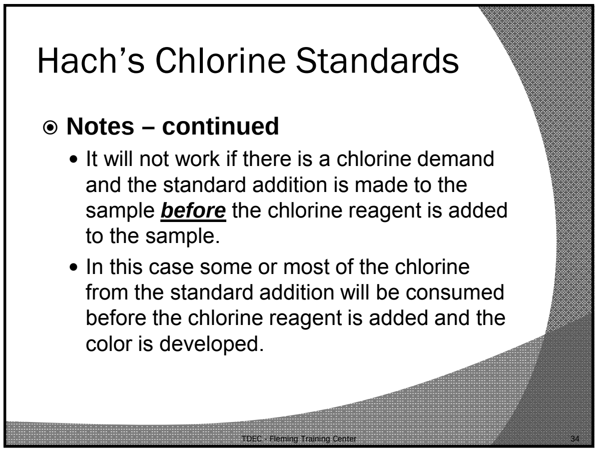
Hach's Chlorine Standards

- Therefore, the addition of 0.1 mL ampule standard should increase the chlorine content by 0.26 mg/L .
- If less is recovered, begin a systematic check to locate and correct the problem.



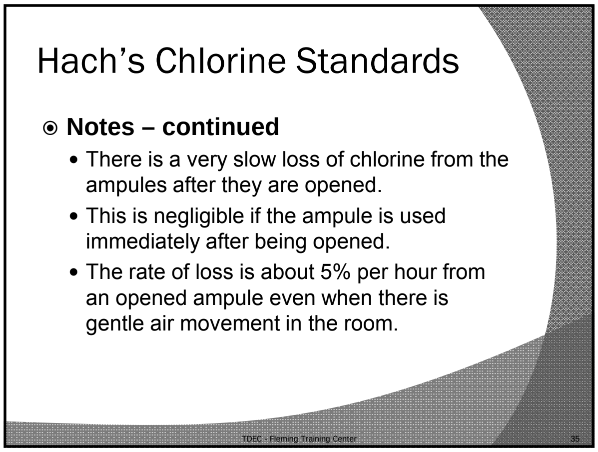
Hach's Chlorine Standards

- **Notes**
  - The use of the Free Chlorine Standards for standard additions performs entirely satisfactorily when there is a chlorine residual ***already*** in the sample and the standard additions are made to the sample ***after*** the chlorine reagent (DPD) is already in the sample.
  - The system will work quite well even if the sample has a small chlorine demand because the free chlorine will react with the chlorine reagent to produce the chlorine color before it is consumed by the chlorine demand in the sample.



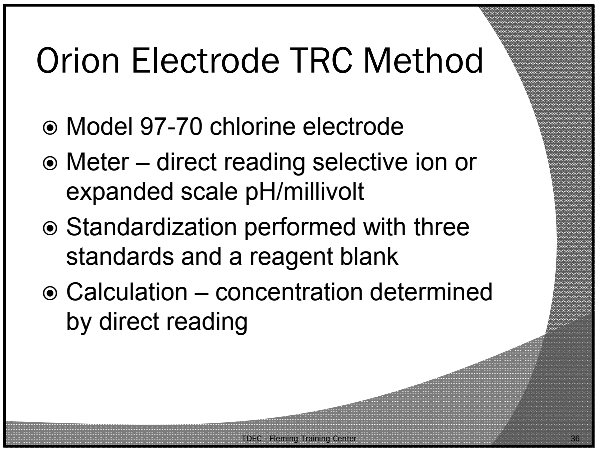
Hach's Chlorine Standards

- **Notes – continued**
  - It will not work if there is a chlorine demand and the standard addition is made to the sample ***before*** the chlorine reagent is added to the sample.
  - In this case some or most of the chlorine from the standard addition will be consumed before the chlorine reagent is added and the color is developed.



Hach's Chlorine Standards

- **Notes – continued**
  - There is a very slow loss of chlorine from the ampules after they are opened.
  - This is negligible if the ampule is used immediately after being opened.
  - The rate of loss is about 5% per hour from an opened ampule even when there is gentle air movement in the room.



Orion Electrode TRC Method

- Model 97-70 chlorine electrode
- Meter – direct reading selective ion or expanded scale pH/millivolt
- Standardization performed with three standards and a reagent blank
- Calculation – concentration determined by direct reading

Amperometric Titration

- Most sensitive and most complex method
- Least affected by interferences
- Training in proper determination technique
- Titrant initially verified and periodically checked
- Fresh titrant and proper buret
- Titrant storage – dark and cool

Amperometric Titration

- Apparatus
  - Amperometric Titrator (Wallace & Tiernan)
  - Buret with 0.01 mL increments
- Reagents
  - Phenylarsine oxide titrant, 0.00564N
  - Potassium Iodide solution (KI solution)
  - Acetate Buffer solution

Amperometric Titration - Procedure

- Fill burette with 0.0056N phenylarsine oxide solution
- Measure 200 mL of sample into the cell and place in the holder on the titrator
- Add 1 mL KI solution (5% solution)
- Add 1 mL acetate buffer solution
- Turn on stirrer and adjust control knob until the meter reads the maximum on the scale

Amperometric Titration - Procedure

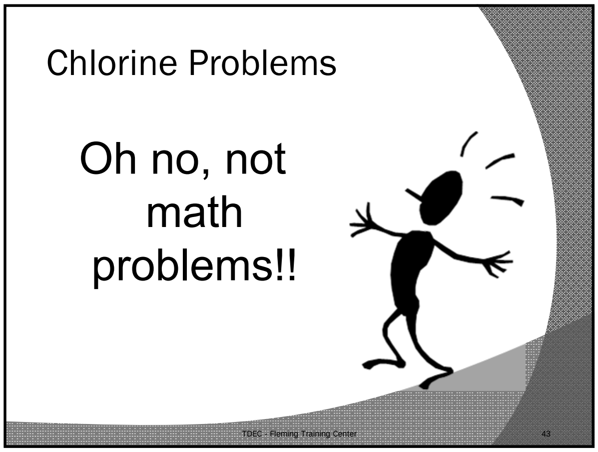
- Add phenylarsine oxide in 0.01 mL increments.
  - This should cause the meter reading to deflect downward.
  - Adjust the control knob as needed to keep the pointer on the scale.
  - The end-point is reached when the addition of titrant no longer results in a downward deflection.

Amperometric Titration - Procedure

- Read the burette, subtracting the amount of the last addition (which did not cause a downward deflection).
  - The burette reading in mL equals the free chlorine residual in mg/L.

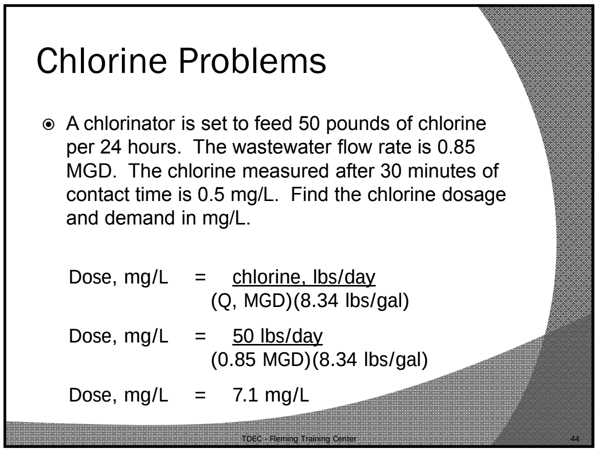
Common Deficiencies

- Sampling and analyses times were not documented for field parameters
- Standards weren't analyzed to verify the accuracy of the chlorine meter
- Measuring free residual chlorine
- Non-approved method being used to measure TRC
- TRC was being measured on the composite sample



Chlorine Problems

Oh no, not math problems!!



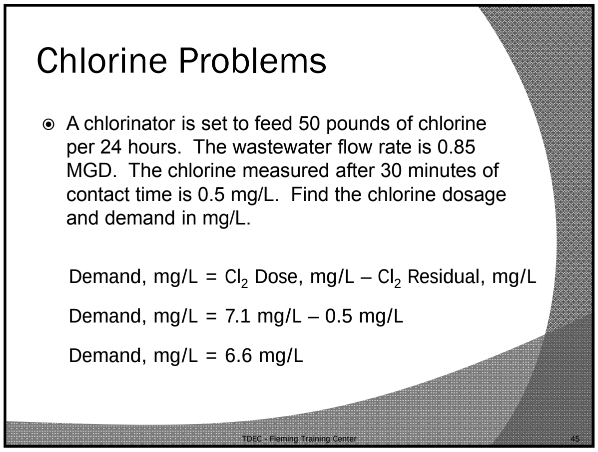
Chlorine Problems

- A chlorinator is set to feed 50 pounds of chlorine per 24 hours. The wastewater flow rate is 0.85 MGD. The chlorine measured after 30 minutes of contact time is 0.5 mg/L. Find the chlorine dosage and demand in mg/L.

$$\text{Dose, mg/L} = \frac{\text{chlorine, lbs/day}}{(\text{Q, MGD})(8.34\text{ lbs/gal})}$$

$$\text{Dose, mg/L} = \frac{50\text{ lbs/day}}{(0.85\text{ MGD})(8.34\text{ lbs/gal})}$$

$$\text{Dose, mg/L} = 7.1\text{ mg/L}$$



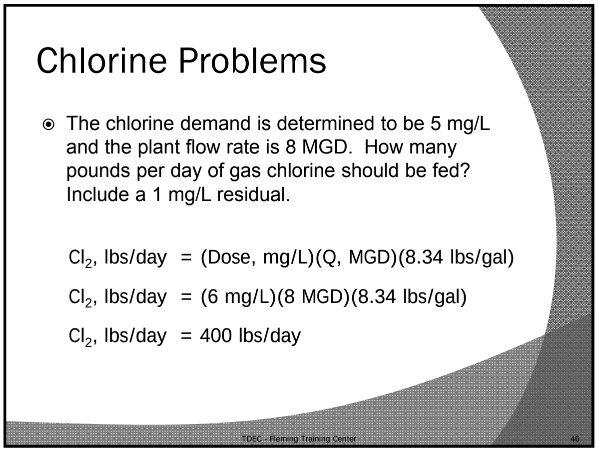
Chlorine Problems

- A chlorinator is set to feed 50 pounds of chlorine per 24 hours. The wastewater flow rate is 0.85 MGD. The chlorine measured after 30 minutes of contact time is 0.5 mg/L. Find the chlorine dosage and demand in mg/L.

Demand, mg/L = Cl_2 Dose, mg/L – Cl_2 Residual, mg/L

Demand, mg/L = 7.1 mg/L – 0.5 mg/L

Demand, mg/L = 6.6 mg/L



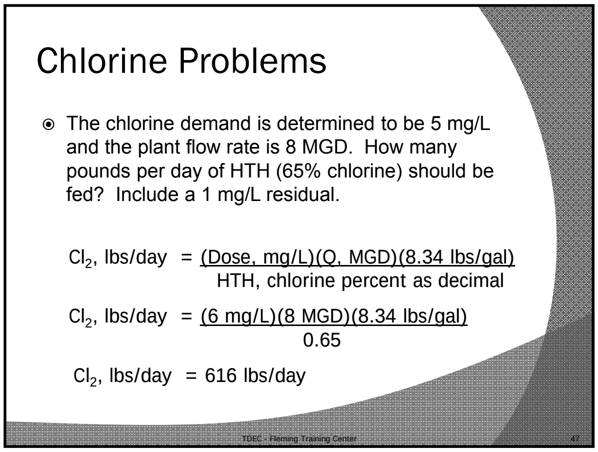
Chlorine Problems

- The chlorine demand is determined to be 5 mg/L and the plant flow rate is 8 MGD. How many pounds per day of gas chlorine should be fed? Include a 1 mg/L residual.

Cl_2, lbs/day = (Dose, mg/L)(Q, MGD)(8.34 lbs/gal)

Cl_2, lbs/day = (6 mg/L)(8 MGD)(8.34 lbs/gal)

Cl_2, lbs/day = 400 lbs/day



Chlorine Problems

- The chlorine demand is determined to be 5 mg/L and the plant flow rate is 8 MGD. How many pounds per day of HTH (65% chlorine) should be fed? Include a 1 mg/L residual.

$$Cl_2\text{, lbs/day} = \frac{(\text{Dose, mg/L})(\text{Q, MGD})(8.34\text{ lbs/gal})}{\text{HTH, chlorine percent as decimal}}$$

$$Cl_2\text{, lbs/day} = \frac{(6\text{ mg/L})(8\text{ MGD})(8.34\text{ lbs/gal})}{0.65}$$

$$Cl_2\text{, lbs/day} = 616\text{ lbs/day}$$

Chlorine, Total

DOC316.53.01027

USEPA[1] DPD Method[2] **Method 8167**

(0.02 to 2.00 mg/L) **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For testing residual chlorine and chloramines in water, wastewater, estuary water and seawater; USEPA-accepted[1] for reporting for drinking and wastewater analyses.

[1] Procedure is equivalent to USEPA method and Standard Method 4500-Cl G for drinking water and wastewater analyses.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Powder pillows			AccuVac Ampuls	
	Sample cell	Cell orientation	Adapter	Sample cell	Adapter
DR 5000	2495402	Fill line faces user	A23618	2427606	A23618
DR 2800	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR 2700	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR/2500	2427606	—	—	2427606	—
DR/2400	2427606	—	—	2427606	—

Before starting the test:

Samples must be analyzed immediately and cannot be preserved for later analysis
If the test overranges, dilute the sample with a known volume of high quality, chlorine demand-free water and repeat the test. Some loss of chlorine may occur due to the dilution. Multiply the result by the dilution factor. Or, analyze samples with high chlorine concentrations directly without dilution by using Method 10070, Chlorine, Total HR.
For chloramination disinfection control, use Method 10172, Chloramine (Mono), Low Range (program number 66) or High Range (program number 67).
After adding reagent a pink color will develop.
The SwifTest Dispenser[1] for Total Chlorine can be used in place of the powder pillow in step 3.
An empty AccuVac ampule can be used as a blank in place of the sample cell in step 2.

[1] *Optional reagents and apparatus.*

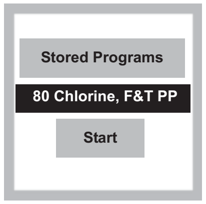
Chlorine, Total

Collect the following items:

Description	Quantity
Powder Pillow Test:	
DPD Total Chlorine Reagent powder pillow, 10-mL	1
Sample Cells (see *Instrument-specific information*)	2
AccuVac Test:	
Collect at least 40 mL of sample in a 50-mL beaker	40 mL
DPD Total Chlorine Reagent AccuVac® Ampul	1
Beaker, 50-mL '	1
Sample Cell (see *Instrument-specific information*)	1

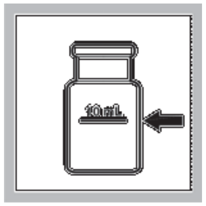
See *Consumables and replacement items* for reorder information.

DPD method for powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. Fill a sample cell with 10 mL of sample.

3. Prepared Sample:

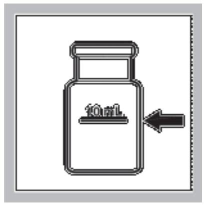
Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell.

Swirl the sample cell for 20 seconds to mix.

4. Start the instrument timer.

A three-minute reaction period will begin. Perform steps 5 and 6 during this time period.

DPD method for powder pillows (continued)



5. Blank Preparation: Fill a second sample cell with 10-mL of sample.

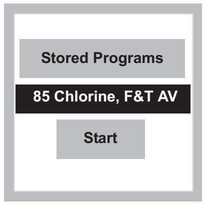
6. Wipe the blank sample cell and insert it into the cell holder.

ZERO the instrument. The display will show: 0.00 mg/L Cl_2

7. Within three minutes after the timer expires, wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L Cl_2.

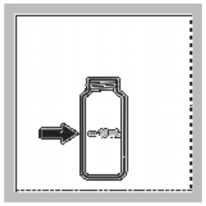
DPD method for AccuVac Ampuls



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.



2. Blank Preparation: Fill a sample cell with 10-mL of sample.

3. Prepared Sample:

Fill a DPD Total Chlorine Reagent AccuVac® Ampul with sample. Keep the tip immersed while the Ampul fills completely.

4. Quickly invert the Ampul several times to mix. Wipe off any liquid or fingerprints.

DPD method for AccuVac Ampuls (continued)

5. Start the instrument timer.

A three-minute reaction period will begin. Perform steps 6 and 7 during this time period.

6. Wipe the blank sample cell and insert it into the cell holder.

ZERO the instrument. The display will show: 0.00 mg/L Cl_2

7. Within three minutes after the timer expires, wipe the AccuVac Ampul and insert it into the cell holder.

READ the results in mg/L Cl_2.

Interferences

Table 2 Interfering substances and levels

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sodium hydroxide[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Alkalinity	Greater than 300 mg/L $CaCO_3$. May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid[1]. Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Bromine, Br_2	Interferes at all levels
Chlorine Dioxide	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1000 mg/L as $CaCO_3$
Iodine, I_2	Interferes at all levels
Manganese, Oxidized (Mn^{4+}, Mn^{7+}) or Chromium, Oxidized (Cr^{6+})	**1.** Adjust sample pH to 6–7. **2.** Add 3 drops potassium iodide[1] (30 g/L) to a 25-mL sample. **3.** Mix and wait one minute. **4.** Add 3 drops sodium arsenite[1, 2] (5 g/L) and mix. **5.** Analyze 10 mL of the treated sample as described in the procedure. **6.** Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or Highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid[1], 1.000 N) or base (Sodium Hydroxide[1], 1.00 N).

[1] See *Optional reagents and apparatus*.

[2] Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). Reference the current MSDS for more information on proper disposal of these materials.

Sample collection, preservation and storage

- Analyze samples for chlorine immediately after collection. Chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of chlorine in water.
- Avoid plastic containers since these may have a large chlorine demand.
- Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to l liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.
- Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.
- A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark.
- Perform the chlorine analysis immediately.

Accuracy check

Required for accuracy check:

- Chlorine Voluette® Ampule Standard, 25–30 mg/L Cl_2.
- TenSette Pipet

Standard additions method (sample spike)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu:

Instrument	Navigate to:
DR 5000	**OPTIONS>MORE>STANDARD ADDITIONS**
DR 2800	**OPTIONS>MORE>STANDARD ADDITIONS**
DR 2700	**OPTIONS>MORE>STANDARD ADDITIONS**
DR/2500	**OPTIONS>STANDARD ADDITIONS**
DR/2400	**OPTIONS>STANDARD ADDITIONS**

3. Default values for standard concentration, sample volume and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open a LR Chlorine Voluette Ampule Standard, 25–30 mg/L Cl_2.
5. Prepare three sample spikes. Fill three mixing cylinders with 10 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples and mix each thoroughly.

   Note: *For AccuVac® Ampuls, fill three mixing cylinders with 50-mL of sample and spike with 0.4 mL, 0.8 mL and 1.2 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers*. Analyze each standard addition sample as described in the procedure above. Accept each standard additions reading by pressing Read. Each addition should reflect approximately 100% recovery.*

6. Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by pressing Read. Each addition should reflect approximately 100% recovery.
7. After completing the sequence, press GRAPH to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press IDEAL LINE to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
80	DR 5000	1.25 mg/L Cl_2	1.23–1.27 mg/L Cl_2	0.02 mg/L Cl_2
	DR 2800	1.25 mg/L Cl_2	1.23–1.27 mg/L Cl_2	0.02 mg/L Cl_2
	DR 2700	1.25 mg/L Cl_2	1.23–1.27 mg/L Cl_2	0.02 mg/L Cl_2
	DR/2500	1.07 mg/L Cl_2	1.05–1.09 mg/L Cl_2	0.02 mg/L Cl_2
	DR/2400	1.07 mg/L Cl_2	1.05–1.09 mg/L Cl_2	0.02 mg/L Cl_2

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
85	DR 5000	1.25 mg/L Cl_2	1.21–1.29 mg/L Cl_2	0.02 mg/L Cl_2
	DR 2800	1.25 mg/L Cl_2	1.21–1.29 mg/L Cl_2	0.02 mg/L Cl_2
	DR 2700	1.25 mg/L Cl_2	1.21–1.29 mg/L Cl_2	0.02 mg/L Cl_2
	DR/2500	1.07 mg/L Cl_2	1.03–1.11 mg/L Cl_2	0.02 mg/L Cl_2
	DR/2400	1.07 mg/L Cl_2	1.03–1.11 mg/L Cl_2	0.02 mg/L Cl_2

Summary of method

Chlorine can be present in water as free chlorine and as combined chlorine. Both forms can exist in the same water and be determined together as the total chlorine. Free chlorine is present as hypochlorous acid and/or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride and other chloro derivatives. The combined chlorine oxidizes iodide in the reagent to iodine. The iodine and free chlorine reacts with DPD (N,N-diethyl-p-phenylenediamine) to form a pink color which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run a free chlorine test. Subtract the results of the free chlorine test from the total chlorine test to obtain the combined chlorine concentration. Test results are measured at 530 nm.

Section 14
Bacteriological

Bacteriological Analysis

Intermediate Wastewater Lab

Coliform Bacteria

- MPN of coliform bacteria are estimated to indicate the presence of bacteria originating from the intestines of warm-blooded animals
- Coliform bacteria are generally considered harmless
  - But their presence may indicate the presence of pathogenic organisms

Coliform Bacteria

- Comprises all the aerobic and facultative anaerobic gram negative, nonspore-forming, rod-shaped bacteria that ferment lactose within 48 hours at 35°C
- Coliform bacteria can be split into fecal and non-fecal groups
- The fecal group can grow at higher temperatures (45 °C) than the non-fecal coliforms

Approved Methods

- Coliform (fecal)
  - Number per 100 mL
    - Membrane filtration
- E. coli
  - Number per 100 mL
    - Multiple tub/multiple well (Colilert®)
    - Membrane filtration
      - m-ColiBlue24®
      - Modified mTEC agar

Fecal Coliform

Discussion

- Most probable numbers of coliform bacteria are estimated to indicate the presence of bacteria of fecal origin
- Coliforms considered harmless
- Presence may indicate presence of disease-causing organisms
- Coliforms: gram negative, nonspore forming rod-shaped bacteria that ferment lactose at 35°C
- Fecal coliforms: grow at higher temperature (44.5°C)

Summary of Method

- A 100 mL volume of sample is filtered through a 47-mm membrane filter using standard techniques.
- Filter is transferred to a 50-mm petri plate containing an absorbent pad saturated with mFC Broth.
- Invert filter and incubate at 44.5±0.2°C for 24 hrs.
- Count blue colonies.

Interferences

- No interferences
- Excess particulates may cause colonies to grow together on a crowded filter or slow the sample filtration process.

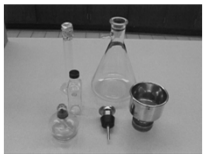
Safety

- Follow standard safety practices appropriate to microbiological laboratories.
- Materials suspected of containing viable bacteria should be decontaminated using an autoclave or by using an appropriate disinfectant before discarding.

Equipment

- Water bath or air incubator operating at 44.5±0.2°C
- Vacuum pump
- UV sterilizer or boiling water bath
- 10-15 X dissecting microscope; should have fluorescent illuminator
- Alcohol burner

Supplies and Glassware



- Sterile graduated cylinder
- Sterile pipets
- Sterile MF filtration flask
- Sterile dilution water
- Sterile sample vessels
- Samples containing chlorine must be treated with 3% sodium thiosulfate solution
- mFC Broth

Sampling

- Clean, sterilized borosilicate glass or plastic bottles or sterile plastic bags.
- Leave ample air space for mixing.
- Collect samples representative of wastewater tested.
- Use aseptic techniques; avoid sample contamination.
- Test samples as soon as possible.

Sampling

- Maximum hold time is 8 hrs at < 10°C
- Ideal sample volume yields 20-60 colonies
- Samples <20 mL, add 10 mL sterile dilution water to filter funnel before applying vacuum.
- Sterilize funnel between samples.

Fecal Data Analysis

- Visually determine colony counts on membrane filters.
- Verify using 10-15 X binocular wide-field microscope.
- Fecal coliforms appear blue.

Fecal Data Interpretation

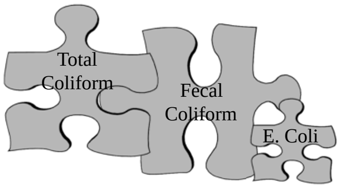
- Incubation time is 24 ± 2 hrs.
- Fecal coliform density reported as number of colonies per 100 mL of sample.
- NPDES permit limit: monthly average of 200/100 mL; daily maximum of 1000/100 mL.

Escherichia coli (E.coli)

Colilert

Family Portrait

- Indicators of water contamination



Techniques for Measuring

- Most Probable Number (MPN)
- Membrane Filter

Colilert® & Colilert-18®

MPN Method

- Add substrate to a 100 mL sample
- If making dilutions, use sterile DI water, not sterile buffered water.

Colilert® & Colilert-18®

- Shake sample vigorously. Wait for bubbles to dissipate.
- Pour into QuantiTray.

Colilert® & Colilert-18®

- Seal sample in Quanti-Tray
- Incubate at 35±0.5°C for 18 hrs (Colilert-18) OR 24 hrs (Colilert)

Colilert® & Colilert-18®

- Examine tray for appropriate color change
- Yellow is an indicator of total coliforms

Left: The 97 well QuantiTray 2000 will count up to 2419 cfu without dilution.
Right: The 51 well QuantiTray will count up to 200 cfu without dilution.

Colilert® & Colilert-18®

- Examine positive total coliform for fluorescence using a UV light in a dark environment
- Fluorescence is a positive indicator for E. coli
- Calculate MPN value according to the table provided with the QuantiTray

Escherichia coli (E.coli)

Modified mTEC Agar with Membrane Filtration

EPA Method 1603

- Membrane Filter – modified mTEC agar
- Filter sample dilutions through a 47mm diameter sterile, white, grid marked filter (0.45µm pore size)
- Place sample in a petri dish with modified mTEC agar

Method 1603

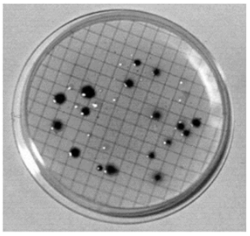
- Invert dish and incubate for 35± 0.5°C for 2 hours
  - Resuscitates injured or stressed bacteria
- Then incubate at 44.5± 0.2°C for 22 hours
- After incubation, remove the plate from the water bath or dry air incubator
- Daily QC adds quite a bit to this test

Method 1603

- Count and record the number of red or magenta colonies (verify with stereoscopic microscope)
- See the USEPA microbiology methods manual, Part II, Section C, 3.5, for general counting rules

Method 1603

Modified mTEC



Count magenta colonies as *E. coli*. These are easily discerned from non-target colonies which are clear or beige.

Method 1603

- QC Tests:
  - Initial precision and recovery
  - Ongoing precision and recovery
  - Matrix spike
  - Negative control
  - Positive control
  - Filter sterility check
  - Method blank
  - Filtration blank
  - Media sterility check

Method 1603

- Initial precision and recovery
  - Should be performed by each lab before the method is used for monitoring field samples
- Ongoing precision and recovery
  - Run after every 20 field and matrix spike samples or one per week that samples are analyzed
- Matrix spike
  - Run 1 per 20 samples

Method 1603

- Negative control
  - Should be analyzed whenever a new batch of media or reagents is used
- Positive control
  - Should be analyzed whenever a new batch of media or reagents is used
- Filter sterility check
  - Place at least one membrane filter per lot of filters on a tryptic soy agar (TSA) plate and incubate for 24 ± 2 hours at 35ºC ± 0.5 ºC .
  - Absence of growth indicates sterility of the filter.
  - Run daily.

Method 1603

- Method blank
  - Filter a 50-mL volume of sterile buffered dilution water and place on a modified mTEC agar plate and incubate.
  - Absence of growth indicates freedom of contamination from the target organism.
  - Run daily.
- Filtration blank
  - Filter a 50-mL volume of sterile buffered dilution water and place on a TSA plate and incubate at just at 35ºC ± 0.5 ºC for 24 ± 2 hours .
  - Absence of growth indicates sterility of the buffer and filtration assembly.
  - Run daily.

Method 1603

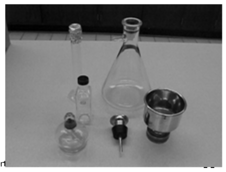
- Media sterility check
  - The lab should test media sterility by incubating one unit (tube or plate) from each batch of medium (TSA, modified mTEC and verification media) as appropriate and observing for growth.
  - Absence of growth indicates media sterility.
  - Run daily.

Escherichia coli (E.coli)

m-ColiBlue24® with Membrane Filtration

m-ColiBlue24®

- Membrane Filter
- Filter sample dilutions through a 47mm diameter sterile, white, grid marked filter (0.45µm pore size)

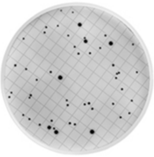


m-ColiBlue24®

- Place sample in a petri dish with absorbent pad containing 2 mL mColiBlue 24 broth
- Invert dish and incubate at 35± 0.5°C for 24 ±2 hours

m-ColiBlue24®

- After incubation, remove the plate from the water bath or dry air incubator
- Count and record the number of blue colonies (verify with stereoscopic microscope)
- See the USEPA microbiology methods manual, Part II, Section C, 3.5, for general counting rules



E. coli Data Analysis

- Maximum sample hold time: 6 hrs
- Samples and equipment known or suspected to have viable E. coli attached or contained must be sterilized prior to disposal.

E. coli Data Interpretation

- Permit limit: 126 colonies/100 mL monthly average
- For MF method:
  - Select sample volumes to produce 20-80 colonies on the membranes.
  - Run minimum of 3 dilutions.
  - Must use sterile buffered water for dilutions and to rinse filtration unit.

Expected Reactions of Various Microorganisms

- Total coliforms will produce a red colony
  - Enterobacter species
    - *E. cloacae*
    - *E. aerogenes*
  - Klebsiella species
    - *K. pneumoniae*
  - Citrobacter species
    - *C. freundii*
- *Escherichia coli* will produce a blue colony
  - *E. coli* O157:H7 will not produce a blue colony, but will grow as a red colony

Expected Reactions of Various Microorganisms

- Known negative reaction (no growth) after 24-25 hours
  - *Pseudomonas aeruginosa*
    - *Variable reaction may be positive for total coliform when incubated longer than 25 hours*
  - *Proteus vulgaris*
  - *Aeromonas hydrophila*

Expected Reactions of Various Microorganisms

- Some strains of the following microorganisms are known to produce a false-positive total coliform reaction (a red colony, but not a true total coliform)

- *Serratia species*
- *Hafnia alvei*
- *Vibrio fluvialis*
- *Aeromonas species*
- *Proteus vulgaris*
- *Providencia stuartii*
- *Yersinia enterocolitica*
- *Leclercia adecarboxylata*
- *Ewingella americana*
- *Staphylococcus species*
- *Proteus mirabilis*

M-ColiBlue24® Trouble-Shooting Guide, Hach Company, www.Hach.com

E. coli Information

- For Colilert ®: IDEXX Laboratories, www.idexx.com
- For mTEC Agar and mColiBlue-24® media: Hach Company, www.Hach.com
- EPA Method 1603: E.coli In Water By Membrane Filtration Using Modified-Thermotolerant Escherichia coli Agar (Modified mTEC), September 2002, EPA-821-R-02-023

All Bacteriological Checks

- Temperatures are documented daily
- Thermometers are certified at least annually against NIST thermometers
- Reagents for storage requirements and expiration dates

All Bacteriological Checks

- E. coli colonies identified correctly
- Calculations are correct
- Holding Times are met
  - Sample collection
  - Analysis start
  - End times

Membrane Filter Counts

Within Acceptable Limits

$$\text{Count per 100 mL} = \frac{\text{Number of colonies}}{\text{Vol. of sample filtered (mL)}} \times 100$$

- Assume that filtration of volumes 50, 15, 5, 1.5 and 0.5 mL produced colony counts of 200, 110, 40, 10 and 5 respectively
- You do not need to count the colonies on all the filters. Select the membrane filters (MF's) with 20-60 Fecal Coliforms and 20-80 E. coli colonies

Within Acceptable Limits

$$\text{Count per 100 mL} = \frac{\text{Number of colonies}}{\text{Vol. of sample filtered (mL)}} \times 100$$

- After selecting the best MF's with a 40 colony count, you apply the general formula as follows

$$\text{Count per 100 mL} = \frac{40}{5} \times 100 = 800/100 \text{ mL}$$

More Than One Acceptable Count

- If there are acceptable counts on replicate plates, carry counts independently to final reporting units, then calculate the arithmetic mean of these counts to obtain the final reported value

More Than One Acceptable Count

- For example, 1 mL volumes produce coliform counts of 26 and 36 or counts of 2600 and 3600/100 mL

$$\frac{2600 + 3600}{2} = 3100/100 \text{ mL}$$

More Than One Acceptable Count

- If more than one dilution, independently carry counts to final reporting units, then average for final reported value

More Than One Acceptable Count

- For example, assume that volumes of 0.3, 0.05, 0.03 and 0.01 mL produced coliform colony counts of TNTC (Too Numerous To Count), 55, 30 and 8 respectively.
- In this example, two volumes, 0.05 and 0.03 produce colonies in the acceptable counting range

More Than One Acceptable Count

- Independently carry each MF count to a count per 100 mL

$\frac{55}{0.05} \times 100 = 110{,}000/100$ mL $\frac{30}{0.03} \times 100 = 100{,}000/100$ mL

- Then calculate the arithmetic mean of these counts to obtain the final reported value

$$\frac{110{,}000 + 100{,}000}{2} = 105{,}000/100 \text{ mL}$$

If All MF Counts are Below the Lower Limit

- Select the most nearly acceptable count
- For example, assume a count in which sample volumes of 1, 0.3 and 0.01 mL produced colony counts of 14, 3 and 0 respectively
- Here, no colony count falls within the recommended limits.
  - Calculate on the basis of the most nearly acceptable plate count, 14, and report with a qualifying remark

$$\frac{14}{1.0} \times 100 = 1400/100 \text{ mL}$$

If All MF Counts are Below the Lower Limit

- Here, no colony count falls within the recommended limits.
  - Calculate on the basis of the most nearly acceptable plate count, 14, and report with a qualifying remark

$\frac{14}{1.0} \times 100 = 1400$ **Report as: estimated 1400/100mL**

If Counts from All MF are Zero

- Calculate using count from largest filtration volume
- For example, sample volumes of 25, 10 and 2 mL produced colony counts of 0, 0 and 0 respectively and no actual calculation is possible, even as an estimated report.

If Counts from All MF are Zero

- Calculate the number of colonies per 100 mL that would have been reported if there had been one colony on the filter representing the largest filtration volume

$\frac{1}{25} \times 100 = 4$ **Report as: < (Less than) 4/100mL**

If All Membrane Counts are Above the Upper Limit

- Calculate the count with the smallest volume filtered
- For example, assume that the volumes 1, 0.3 and 0.01 mL produced colony counts of TNTC, 150 and 110.

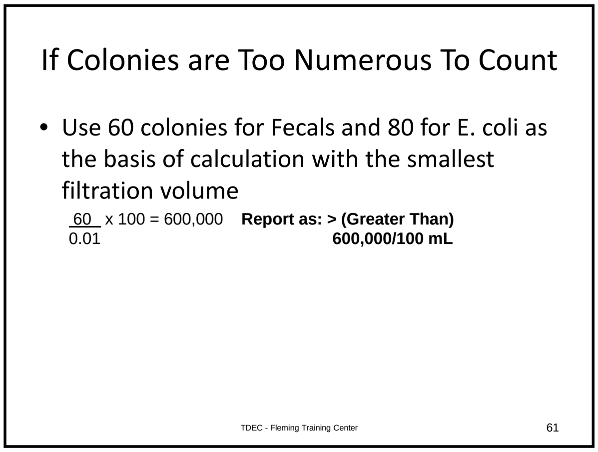
If All Membrane Counts are Above the Upper Limit

- Since all colony counts are above the recommended limit, use the colony count from the smallest sample volume filtered and estimate the count as

$\frac{110}{0.01} \times 100 = 1{,}100{,}000$ **Report as: estimated 1,100,000/100 mL**

If Colonies are Too Numerous To Count

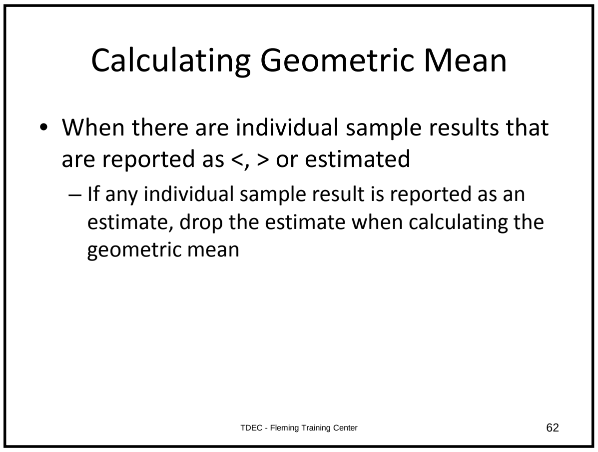
- Use upper limit with smallest filtration volume
- For example, assume that the volumes 1.0, 0.3 and 0.01 mL all produced too many colonies to show separated colonies and that the laboratory bench records showed TNTC



If Colonies are Too Numerous To Count

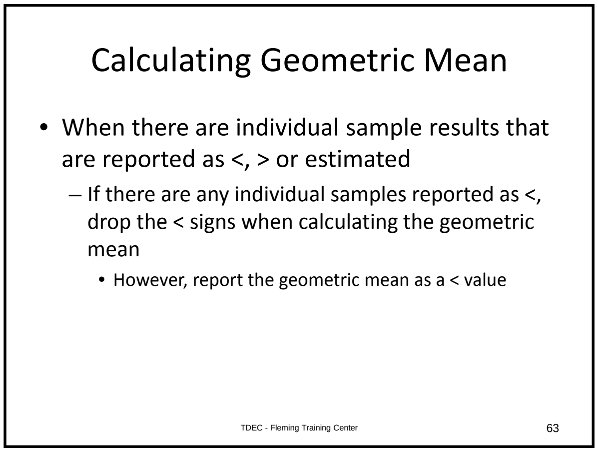
- Use 60 colonies for Fecals and 80 for E. coli as the basis of calculation with the smallest filtration volume

$\frac{60}{0.01} \times 100 = 600{,}000$ **Report as: > (Greater Than) 600,000/100 mL**



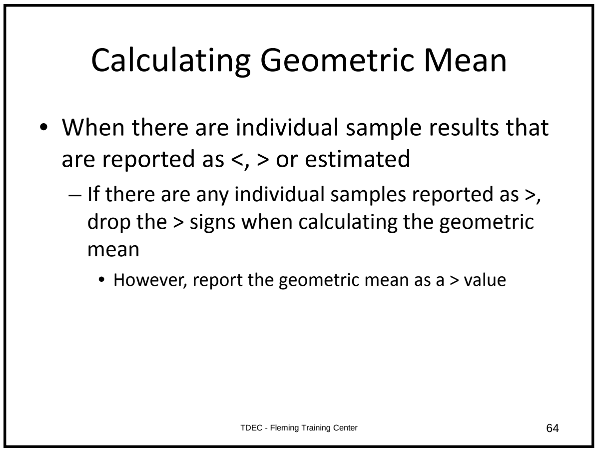
Calculating Geometric Mean

- When there are individual sample results that are reported as <, > or estimated
  - If any individual sample result is reported as an estimate, drop the estimate when calculating the geometric mean



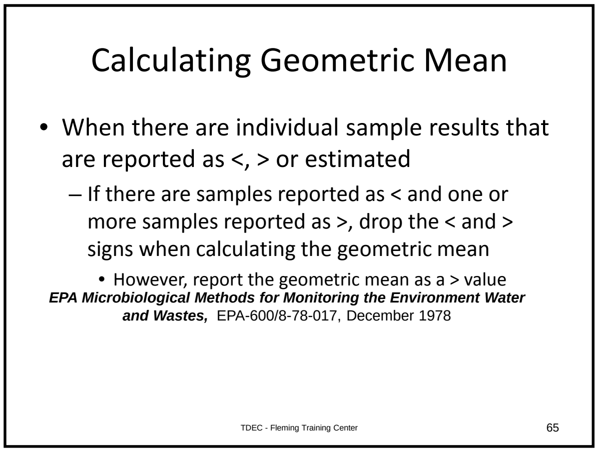
Calculating Geometric Mean

- When there are individual sample results that are reported as <, > or estimated
  - If there are any individual samples reported as <, drop the < signs when calculating the geometric mean
    - However, report the geometric mean as a < value



Calculating Geometric Mean

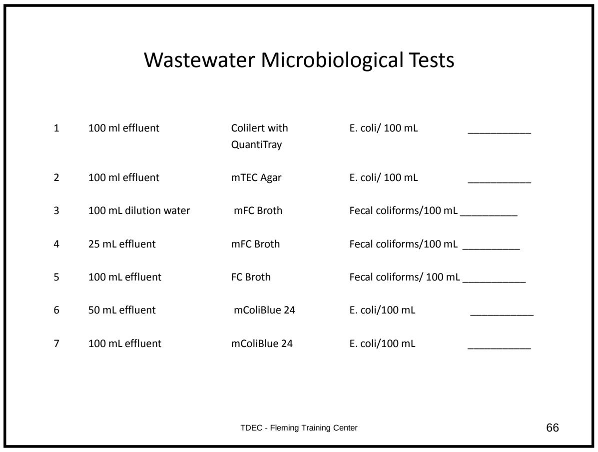
- When there are individual sample results that are reported as <, > or estimated
  - If there are any individual samples reported as >, drop the > signs when calculating the geometric mean
    - However, report the geometric mean as a > value



Calculating Geometric Mean

- When there are individual sample results that are reported as <, > or estimated
  - If there are samples reported as < and one or more samples reported as >, drop the < and > signs when calculating the geometric mean
    - However, report the geometric mean as a > value

EPA Microbiological Methods for Monitoring the Environment Water and Wastes, EPA-600/8-78-017, December 1978



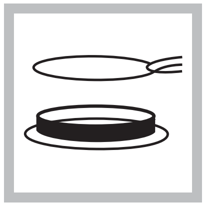
Wastewater Microbiological Tests

1	100 ml effluent	Colilert with QuantiTray	E. coli/ 100 mL	__________
2	100 ml effluent	mTEC Agar	E. coli/ 100 mL	__________
3	100 mL dilution water	mFC Broth	Fecal coliforms/100 mL	__________
4	25 mL effluent	mFC Broth	Fecal coliforms/100 mL	__________
5	100 mL effluent	FC Broth	Fecal coliforms/ 100 mL	__________
6	50 mL effluent	mColiBlue 24	E. coli/100 mL	__________
7	100 mL effluent	mColiBlue 24	E. coli/100 mL	__________

Using m-ColiBlue24 Broth PourRite Ampules

The m-ColiBlue24 Broth can be used to analyze drinking water, bottled water, beverages; surface, well, and groundwater, waste water, recreational waters, and process water for ultrapure, chemical processing and pharmaceutical applications.

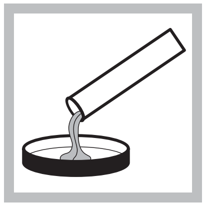
Simultaneous Total Coliform and E.coli Screening Method 10029



1. Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish.

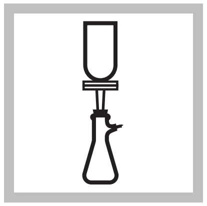
Note: *Do not touch the pad or the inside of the petri dish.*

Note: *To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.*

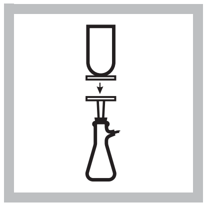


2. Invert ampules two or three times to mix broth. Break open an ampule of m-ColiBlue24 Broth using an ampule breaker. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

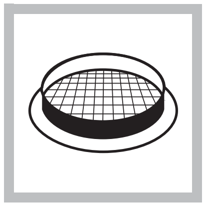
3. Set up the Membrane Filter Apparatus. With sterile forceps, place a membrane filter, grid side up, into the assembly.



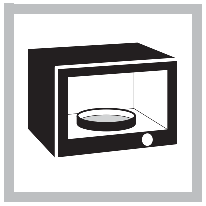
4. Shake the sample vigorously to mix. Pour 100 mL of sample or diluted sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls three times with 20 to 30 mL of sterile buffered dilution water.



5. Turn off the vacuum and lift off the funnel top. Using sterile forceps, transfer the filter to the previously prepared petri dish.



6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.



7. Invert the petri dish and incubate at 35 ±0.5 °C for 24 hours.

8. Remove the petri dish from the incubator and examine the filters for colony growth. Colonies are typically readily visible; however, a stereoscopic microscope or other 10–15X magnifier may be useful. Red and blue colonies indicate total coliforms and blue colonies specifically indicate *E. coli*.

Note: *Sometimes only the center of a colony will be colored. Therefore, a colony with any amount of red color should be counted as red and a colony with any amount of blue should be counted as a blue colony. Red colonies may vary in color intensity. Blue colonies may appear blue to purple. Count all the red and blue colonies as total coliforms. Count all the blue to purple colonies as* E. coli.

Optional Testing of Red Colonies

The m-ColiBlue24 Broth is formulated so that coliforms other than *E. coli* grow as red colonies. The percentage of red colonies that are false positives (non-coliforms) is comparable to the percentage of sheen colonies grown on m-Endo Broth that are false positives (non-coliforms); therefore, confirmation is not required.

A few varieties of the non-coliform bacteria *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. may grow on m-ColiBlue24 Broth and form red colonies. Such bacteria can be readily distinguished from total coliforms by the oxidase test. *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. are oxidase-positive. Total coliforms and *Escherichia coli* are oxidase-negative. If your sample contains high levels of interfering bacteria, you can perform an oxidase test to confirm which red colonies are total coliforms.

Wastewater Lab Class Bacteriological Tests - Group 3

Test	Sample Source	Sample Volume	Media	Colonies / Wells	per 100 mL
Blank	Dilution Water	100 mL	mColiBlue		
E. coli	E. coli	100 mL	mColiBlue		
E. coli	E. coli	100 mL	Colilert QuantiTray (96 well count)		
Fecal	E. coli	100 mL	mFC		

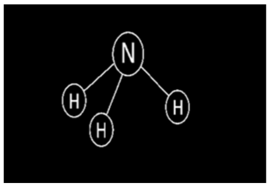
Section 15
Ammonia

Ammonia Nitrogen

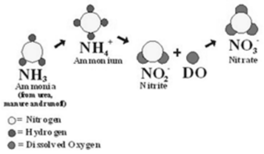
Wastewater Lab

Ammonia Nitrogen

- In domestic wastewater is generally between 10-40 mg/L.
- Primary treatment may increase the ammonia nitrogen slightly due to the composition of some protein compounds during treatment



Ammonia Nitrogen



- During secondary treatment processes, ammonia may be oxidized to nitrite then to nitrate in varying degrees depending on factors such as wastewater temperature, residence time of the microorganisms and oxygen amounts.

Nitrification – The reaction

Nitrosomonas

- $2NH_3 + HCO_3 + 3O_2 \longrightarrow 2HNO_2 + 4H_2O + 2CO_2$

Nitrobacter

- $2HNO_2 + O_2 + 2HCO_3 \longrightarrow 2NO_3 + 2H_2O + 2CO_2$

The Nitrobacter microorganisms use the nitrite in nitrous acid oxidizing it to nitrate.

Ammonia Nitrogen

- Ammonia levels can
  - Increase chlorine demand
  - Cause fish toxicity
  - Increase oxygen demand on receiving water

Forms of Nitrogen in Activated Sludge

- Un-Oxidized Forms of Nitrogen →
  - Nitrogen Gas (N_2)
  - Ammonia
  - Organic Nitrogen including proteins, amino acids, urea, etc..
- Oxidized Forms of Nitrogen
  - Nitrite
  - Nitrate
  - Nitrous Oxide

More on Nitrogen in Activated Sludge

- Total Nitrogen = TKN + NO_2 + NO_3
- TKN (Total Kjeldahl Nitrogen) = NH_3 + Organic Nitrogen
- Rule of thumb:
  - Ammonia makes up Approximately 60% of TKN
  - Organic Nitrogen is typically removed in settled sludge
  - TKN makes up approximately 15% - 20% of the total influent BOD.

Why Nitrify?

- Ammonia can be harmful if discharged.
  - Creates dissolved oxygen sag in receiving stream
  - Toxic to fish and other aquatic life
  - Possible problem for downstream water supplies.
  - Nutrient input (when oxidized).

Toxic to Fish and Aquatic Life

Some Ammonia Effects

Ammonia Levels & Effects	
NH_3 Levels	Effects
0.06 mg/L	Fish can suffer gill damage
0.1 mg/L	Usually indicative of polluted waters
0.2 mg/L	Sensitive fish like trout and salmon begin to die
2.0 mg/L	Ammonia-tolerant fish, like carp, begin to die

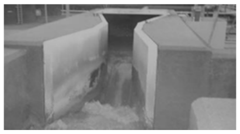
The danger ammonia poses for fish depends on the water's temperature and pH. The higher the pH and temperature, the more toxic the ammonia.

Sources of Ammonia In Wastewater

- Incoming Raw Wastewater (domestic waste)
- Internal Recycle (anaerobic digester supernatant, belt press filtrate) - high
- Septage (high)
- Industrial Sources

How Much Ammonia ???

- Typically, expect influent domestic wastewater to have 25 – 30 mg/l of NH_3-N
- Considered Strong if > 50mg/l NH_3-N
- Septage 150 mg/l



Sample Collection

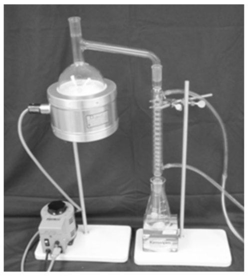
- Collect samples in glass or plastic containers
- Fill sample bottle complete
- If chlorine is present, treat with sodium thiosulfate
  - Add one drop of 0.1N sodium thiosulfate solution for every 0.3 mg/L of chlorine present

Sample Collection

- Analyze as soon as possible
  - Refrigerate at 4°C for samples to be analyzed within 24 hours
  - If this is not possible, preserve the sample with sulfuric acid to pH < 2 and store at 4°C.
    - Samples acidified and cooled may be stored for 28 days
- Before analysis, neutralize the sample to pH 7 with 5N sodium hydroxide

Procedural Concerns

- Ammonia distillation apparatus should be steamed out
- A high & low standard should be carried through the ammonia distillation



Procedural Concerns

- Distillate is caught in boric acid solution for titration or nesslerization
- Distillate is caught in 0.04N H_2SO_4 if using the probe method

Probe Method

- The ammonia electrode measures ammonia gas or ammonium ions in aqueous solutions that have been converted to gas by the addition of a strong base.
- The electrode is a complete electrochemical cell consisting of a glass pH electrode and a reference electrode.
- The gas-permeable membrane separates the sample from a thin layer of electrolyte that is pressed between the pH bulb and the membrane.

Probe Method

- At high pH, ammonium ion is converted to ammonia gas.
- The gas diffuses through the membrane and causes a pH change in the thin layer of electrolyte.
- The potential across the pH glass changes as a result of the pH change and the electrode measures the change in potential.
- The measured pH change is proportional to the ammonia concentration in the solution.

Ammonia Nitrogen Procedure

1) Distillation

- ❋ Prepare Solutions:
  - ∗ Sodium Hydroxide, 6N – Dissolve 24 g of Sodium Hydroxide (NaOH) in75 mL of ammonia-free distilled water and bring to 100 mL.
  - ∗ Boric Acid Solution - Dissolve 20 g boric acid in about 500 mL ammonia-free distilled water in a 500 mL beaker. Add this to a 1000 mL volumetric flask and dilute to 1000 mL. ****Don't need if running probe method.****
  - ∗ Borate Buffer Solution – Dissolve 9.5 g sodium borate in 500 mL of ammonia-free distilled water in a 500 mL beaker. Add this to a 1000 mL volumetric flask. Using a 100 mL graduated cylinder, add 88 mL of 0.1N NaOH to the volumetric flask. Mix well and dilute to 1000 mL.
  - ∗ Sulfuric Acid, 0.04N – Dilute 1.0 mL conc. H_2SO_4 to 1L.
- ❋ Prepare equipment:
  - ∗ Add 500 mL of ammonia-free distilled water to a 1000 mL beaker containing a stir bar. Add 20 mL of borate buffer to beaker. Adjust the pH to 9.5 with 6N NaOH.
  - ∗ Transfer the beaker contents to each distillation flask. Add 5 boiling beads to each flask. Connect all parts of the distillation apparatus.
  - ∗ Turn the cold water and heat elements on. Collect 50 mL portions of distillate in each of two 150 mL beakers. Test for ammonia by using ammonia test strips in each beaker. Discard water. Continue until distillate shows no trace of ammonia on test strip. Turn off the apparatus and allow flasks to cool.
  - ∗ Pour out contents of each distillation flask, collecting boiling beads in a Buchner funnel. Equipment is now ammonia-free.

- Prepare samples:
  - Add boiling beads back to each flask. Measure 500 mL of each sample (***primary clarifier effluent*** and ***plant effluent***) into 1000 mL beakers containing stir bars. Add 25 mL borate buffer to beaker with sample. Adjust pH to 9.5 with 6N NaOH. Add the sample to distillation flask.
    - ***Note: we will be using raw intake instead of primary clarifier effluent from next door.***
- Sample Distillation:
  - Pour 50 mL 0.04 Sulfuric acid to one 500 mL glass stoppered Erlenmeyer flask which are marked at 250 mL (use a graduate cylinder, measure 250 mL and mark flask at water level)
  - Submerge the delivery tube in the sulfuric acid in the flask.
  - Distill the samples at the rate of 6-10 mL/min with delivery tube submerged in sulfuric acid (should take 20 – 33 minutes once distillation starts).
  - Collect distillate up to the 250 mL mark (this is 200 mL distillate and 50 mL sulfuric acid)
  - Set the distillate aside and continue distillation into a 150 mL waste beaker for 3 minutes to cleanse the tube and condenser. Turn off heat element.
  - When cool, add each distillate to a 500 mL volumetric flask. Label the flasks as appropriate. Dilute to the 500 mL mark with ammonia-free distilled water.

Nitrogen, Ammonia

DOC316.53.01235

USEPA[1] Direct Measurement ISE Method[2] **Method 10001**

0.1 to 10.0 mg/L NH_3-N **ISE Electrode**

Scope and Application: For wastewater

[1] USEPA Accepted for reporting wastewater analyses

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, Method 4500NH3E (with distillation). Manual distillation may not be required if comparability data on representative samples in company files show the distillation is not necessary. Manual distillation will be required to resolve any controversies.

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Meter	Electrode
*sens*ion™ 4 meters	5192700
*sens*ion™ 2 meters	5192700

Before starting the test:

Refer to the meter manual for meter operation. Refer to electrode manual for electrode maintenance and care.
Prepare the electrode. Refer to *New electrodes or electrodes stored more than 7 days* and *Electrodes stored 1 to 7 days* for more information.
After every hour of continuous use, place the electrode in the storage solution for 10 minutes to thoroughly recondition. Check with a 10 mg/L NH_3–N standard for accuracy and calibrate if necessary.
At high pH, ammonia solutions lose ammonia to the atmosphere, lowering the concentration. It is important to take measurements as soon as possible after the solution is basic. For most wastewater samples, 1 mL of 10 N NaOH (or equivalent ISA) is sufficient to increase the pH above 11. If in doubt, check the pH with pH paper and add additional NaOH in 0.1 mL increments until the pH exceeds 11.

Collect the following items:

Description	Quantity
Ammonia electrode filling solution	varies
Ammonia electrode storage solution	20 mL
Ammonia nitrogen standard, 100 mg/L NH_3–N	varies
Water, deionized	100 mL
Ammonia ISA solution	2 mL per 100 mL sample
Ammonia electrode, combination BNC	1
Beaker, 150 mL, polypropylene	4
Wash bottle	1
TenSette® pipet, 1.0–10.0 mL	1
*sens*ion 4 laboratory pH/ISE meter or sension 2 portable pH/SE meter	1
Stir bar, 22.2 x 4.76 mm (7/16 x 3/16 in.)	4
Stirrer, electromagnetic, with stand and stir bar	1
25 mL Class A volumetric pipet	1
250 mL Class A volumetric flask	3

See *Consumables and replacement items* for reorder information.

Nitrogen, Ammonia in wastewater method

1. Rinse the electrode with deionized water. Place it in the Ammonia ISE storage solution with the ammonia membrane sensor module on to condition for at least 15 minutes.

2. During the conditioning period prepare three standards. Make a 10-mg/L NH_3–N standard by pipeting 25 mL of 100-mg/L NH_3–N Standard into a 250-mL volumetric flask. Dilute to the mark with ammonia -free deionized water, stopper and thoroughly mix.

3. Prepare a 1.0-mg/L NH_3–N standard by pipeting 25 mL of the 10-mg/L standard into a 250-mL volumetric flask. Dilute to the mark with ammonia-free deionized water, stopper and thoroughly mix.

4. Prepare a 0.1-mg/L NH_3–N standard by pipeting 25 mL of the 1.0-mg/L NH_3–N standard into a 250-mL volumetric flask. Dilute to the mark with ammonia -free deionized water, stopper and thoroughly mix.

Nitrogen, Ammonia in wastewater method (continued)

5. Connect the Ammonia ISE to the BNC connector on the pH/ISE meter. Verify that BNC is selected in Setup 1 of the Setup menu.

6. Turn the meter on. Press **ISE/MV** until the display shows mg/L or other chosen concentration units.

7. Press **CAL**. Use the **ARROW** keys to select the desired units. Press **ENTER** and accept the units.

8. Transfer 100 mL of the 0.1-mg/L NH_3–N standard to a 150-mL beaker. Add a stir bar to the beaker. Put the beaker on a magnetic stirrer and stir at a moderate rate.

9. Remove the electrode from the storage solution. Rinse it with deionized water and blot dry. Put the electrode into the 0.1-mg/L NH_3–N standard.

Make sure no air bubbles are trapped under the tip of the electrode.

10. Pipet 2.0 mL of Ammonia ISA Solution into the standard. Immediately proceed to the next step.

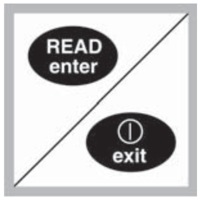
11. Press **READ**. The display will show the value from the previous calibration. Accept the numerical value or use the number keys to change the value to match the concentration of the standard, then press **ENTER** to accept the change.

12. The display will show **Stabilizing...** until the reading is stable. The display will show **Standard 2** and _ _ _ _ or the value of standard 2 from the previous calibration.

Nitrogen, Ammonia in wastewater method (continued)

13. Rinse the electrode with deionized water. Place it in the storage solution for one minute. Repeat steps 8–13 for substituting the 1.0- and 10-mg/L standards.

14. After the last standard is measured, press **EXIT**.



15. The display will show **Store?**. Press **ENTER** to store the calibration or **EXIT** to leave the calibration mode without storing the calibration values.

16. Press **REVIEW**. Use the Up arrow key to scroll to the second slope value. It should be –57 ± 3 mV/decade. If the slope is not –57 ± 3 mV/decade, recalibrate the electrode. If the slope is still incorrect after recalibration, replace the ammonia membrane sensor module.

Press **EXIT** to return to measurement mode.

17. Remove the electrode from the last standard. Rinse it with deionized water and place it in the storage solution.

18. Transfer 100 mL of sample to a 150-mL beaker. Add a stir bar to the beaker. Put the beaker on a magnetic stirrer and stir at a moderate rate.

19. Remove the electrode from the storage solution. Rinse with deionized water and blot dry. Put the electrode into the sample.

20. Pipet 2.0 mL of Ammonia ISA solution into the sample and proceed immediately to the next step.

Nitrogen, Ammonia in wastewater method (continued)

21. Press **READ**. The display will show Stabilizing... until the reading is stable. Record or store the measurement value.

Repeat steps 17–21 for other samples.

Stabilization times will take longer for lower concentrations. A slow downward drift in concentration indicates probable loss of ammonia to the atmosphere. Record the highest value that is stable.

Calibration

Prepare ammonia standard working solutions of 10.0, 1.0 and 0.1 mg/L ammonia nitrogen from a 100-mg/L stock solution. Prepare the standards daily before use. Higher or lower concentration ranges (0.05–1400 mg/L NH_3–N) can be obtained by calibrating the meter with different standard solutions.

Electrode preparation

New electrodes or electrodes stored more than 7 days

Before using a new Ammonia Electrode or an electrode that has been stored dry, remove the protective cap from the end.

1. Unscrew the top cap. Carefully remove the internal glass electrode from the outer body. A white membrane is mounted at the tip of the outer body.
2. Fill the outer body with 3.5 mL of Internal Fill Solution.
3. Rinse the internal glass electrode with deionized water. Blot dry. Return the electrode to the filled outer body. Make sure that the key pin at the top of the internal glass electrode is seated in the slot at the top of the outer body.
4. Reinstall the threaded top cap onto the top of the ammonia electrode body. Finger-tighten the cap until snug. **Do not over-tighten.**

5. Hold the fully assembled electrode securely by one end and shake the electrode with an abrupt downward motion (like shaking the mercury down in a thermometer) to remove bubbles.
6. Place the assembled electrode into the Ammonia Electrode Storage Solution or 1000 mg/L Ammonia Standard for at least 60 minutes.

Electrodes stored 1 to 7 days

- Keep the electrode in 1000 mg/L ammonia standard without Ionic Strength Adjustor (ISA).
- Alternatively, keep the electrode in the Ammonia Electrode Storage Solution.
- Never let the membrane dry out. Cover the storage beaker and electrode body with Parafilm® to prevent solution evaporation.

Electrodes stored between samples

Place the electrode in Ammonia Electrode Storage Solution for at least one minute to initialize the electrode for the next measurement.

Interferences

Distillation prior to ammonia analysis removes all inorganic interferences that complex ammonia.

Table 2 Interfering substances

Interfering substance	Interference level
Amines	Volatile low molecular weight gives a positive interference
Mercury	Complexes with ammonia
Silver	Complexes with ammonia

Sample collection, preservation and storage

- Collect samples in glass or plastic containers of convenient size. Clean new bottles by washing with deionized or distilled water. Fill the sample bottle completely and stopper immediately. Analyze the sample as soon as possible.
- Ammonia may be lost more quickly from samples at temperatures above 50 °C, so it is important to collect samples at less than 40 °C or use a cooling coil between the bottle and sampling point if necessary.
- If chlorine is present, treat the sample immediately with sodium thiosulfate. Add one drop of 0.1 N Sodium Thiosulfate Standard Solution for each 0.3 mg of chlorine present in a one liter sample.
- If prompt analysis is not possible, preserve the sample with 0.8 mL of concentrated sulfuric acid per liter. Use a sension pH meter to be sure the pH of the preserved sample is between 1.5 and 2. Some wastewater samples may require more sulfuric acid to achieve this pH. Store the sample at 4 °C. Samples preserved in this manner may be stored up to 28 days.
- Before analysis, neutralize the sample to pH 7 with 5 N sodium hydroxide. Do not let the pH go above 10. Correct the test results for the volume addition.
- Do not use mercuric chloride as a preservative because ammonia complexes with mercuric ions.

Accuracy check

Standard additions method (sample spike)
To verify measurement accuracy, perform a standard addition spike on the sample. The spike should roughly double the measured concentration without significantly diluting the sample.

To perform a standard addition sample:

1. Use the *Spike volumes for standard additions* table to determine the concentration and volume of standard to spike the sample. The volume of sample transferred must be accurate.
2. Add the amount and concentration specified in the *Spike volumes for standard additions* table to the 100 mL of sample.
3. After adding the standard, proceed with the calculations. Results from 90-110% recovery are typically considered acceptable. Calculate percent recovery as follows:

$$\%\ \text{Recovery} = \frac{100(X_s - X_u)}{K}$$

Where:
X_s = measured value for spiked sample in mg/L

X_u = measured value for unspiked sample adjusted for dilution by the spike, in mg/L

K = known value of the spike in the sample in mg/L

Calculations

1. $X_u = \frac{X_i \times V_u}{V_u + V}$

Where:

X_i = measured value of unspiked sample in mg/L

V_u = volume of separate unspiked portion in mL

V = volume of spike in mL

2. $K = \frac{C \times V}{V_u + V}$

Where:

C = concentration of standard used in spike in mg/L

V = volume of spike in mL

V_u = volume of separate portion before spike in mL

3. Final calculation plugging in X_u and K: $\%\ \text{Recovery} = \frac{100(X_s - X_u)}{K}$

Example:

A sample was analyzed and read 5.0 mg/L NH_3–N. As directed in the *Spike volumes for standard additions* table, a 4.0-mL spike of 100-mg/L NH_3–N standard was added to another 100-mL sample, giving a final standard addition result of 8.75 mg/L.

Calculate the percent recovery as follows:

1. $X_u = \frac{5.0\ \text{mg/L} \times 100\ \text{mL}}{100\ \text{mL} + 4\ \text{mL}} = 4.81\ \text{mg/L}$

2. $K = \frac{100\ \text{mg/L} \times 4\ \text{mL}}{100\ \text{mL} + 4\ \text{mL}} = 3.85\ \text{mg/L}$

3. $\%R = \frac{100 \times (X_s - X_u)}{K} = \frac{100 \times (8.75 - 4.81)}{3.85} = 102.3\ \%\ \text{Recovery}$

Table 3 Spike volumes for standard additions

Measured Sample Concentration (mg/L)	Measured Sample Volume (mL)	Standard Concentration (mg/L)	Standard Volume (mL)
0.1–0.3	100	100	0.2
0.3–0.5	100	100	0.4
0.5–0.7	100	100	0.6
0.7–0.9	100	100	0.8
0.9–1.1	100	100	1.0
1.0–3.0	100	100	2.0
3.0–6.0	100	100	4.0
6.0–10.0	100	100	8.0

Method performance

Instrument	Standard	Precision 95% Confidence Limits of Distribution
sension 4[1]	0.80 mg/L	0.78–0.82 mg/L
sension 2[1]		

[1] With a default stabilization criteria of 0.5 mV/min.

Summary of method

The ammonia electrode measures ammonia gas or ammonium ions in aqueous solutions that have been converted to gas by the addition of a strong base. The electrode is a complete electrochemical cell consisting of a glass pH electrode and a reference electrode.

The gas-permeable membrane separates the sample from a thin layer of electrolyte that is pressed between the pH bulb and the membrane. At high pH, ammonium ion is converted to ammonia gas.

The gas diffuses through the membrane and causes a pH change in the thin layer of electrolyte. The potential across the pH glass changes as a result of the pH change and the electrode measures the change in potential. The measured pH change is proportional to the ammonia concentration in the solution.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Ammonia Electrode Filling Solution	varies	60 mL	4447226
Ammonia Electrode Storage Solution	20 mL	500 mL	2541249
Ammonia Nitrogen Standard, 100 mg/L NH_3-N	100 mL	500 mL	2406549
Ammonia ISA Solution	2 mL/100 mL sample	500 mL	2824349
Water, deionized	100 mL	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Ammonia Electrode	1	each	5192700
Beaker, 150 mL, polypropylene	4	each	108044
Bottle, wash, 500 mL	1	each	62011
Flask, volumetric, Class A, 250 mL	3	each	1457446
sension 4 Laboratory pH/ISE Meter or *sension* 2 pH/ISE (portable)	1	each	5177500
Stir Bar, 22.2 x 4.76 mm	4	each	4531500
TenSette® Pipet, 1.0–10.0 mL	1	each	1970010
Pipet tips for 1970010 TenSette Pipet	varies	50/pkg	2199796
Class A 25 mL volumetric pipet	1	each	1451540
Safety bulb pipet filler	1	each	1418900
Select one based on available voltage:			
Stirrer, electromagnetic 115 V, with stand and stir bar	1	each	4530001
Stirrer, electromagnetic 230 V, with stand and stir bar	1	each	4530002

Optional reagents

Description	Unit	Catalog number
Ammonia Nitrogen Standard Solution 1000 mg/L NH_3-N	1 L	2354153
pH Paper, pH 9.0-12.0	5 rolls/pkg	38533
Sulfuric Acid, concentrated	500 mL	97949

Optional apparatus

Description	Unit	Catalog number
Air Gap Assembly	each	5025300
Ammonia Electrode Membrane Modules	4/pkg	5192711
Cylinder, graduated, glass	100 mL	50842
Electrode Washer	each	2704700
Pipet, Volumetric, Class A, 1.00 mL	each	1451535
TenSette® Pipet, 0.1–1.0 mL	each	1970001
Pipet tips for 1970001 TenSette Pipet	50/pkg	2185696
sension 2 Portable pH/ISE Meter	each	5172500

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Updated February 2008, Edition 5

Section 16
Activated Sludge Process Control

Process Control Testing

TENNESSEE DEPARTMENT OF ENVIRONMENT AND CONSERVATION

Activated Sludge Process Control

- What is a process?
  - Continuing operation or development marked by a series of gradual changes that succeed one another in a relatively fixed way and lead toward a particular result or end.

Activated Sludge Process Control

- What is the wastewater process result or end?
  - Clean Water

Treatment Plant Processes

- Flow Monitoring, Equalization
- Screening
- Grit and Grease Removal
- Activated Sludge
  - Aeration Basin, Clarifier, RAS/WAS
- Disinfection

W/W Treatment & Manufacturing

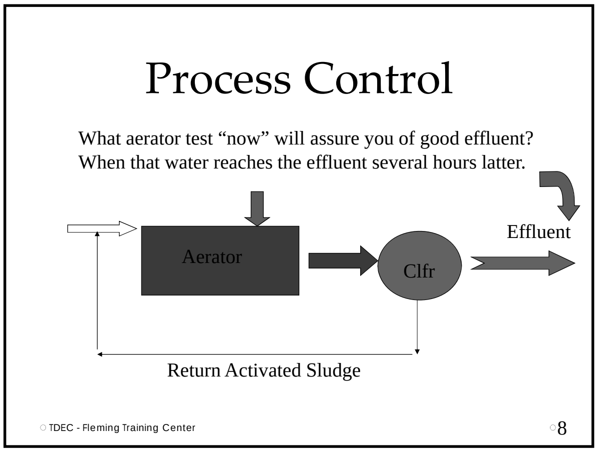
- Manufacturing
  - Many raw materials
  - Manufacturing process
    - Several Steps gradually leading to finished products.
  - Finished product
    - Sausage, cars, pencils; all of which have quality specifications
- Wastewater
  - One raw material
  - Treatment process
    - Several Steps gradually leading to finished products.
  - Finished products
    - Trash, Solid waste rules
    - Clean Water, Permit
    - Biosolids, 503 rule

Common Process Control Methods

- How do you control your activated sludge process?
  - Human senses
    - Visual appearance, odors
  - Process tests
    - Flow, D.O., pH, temp., alkalinity,ORP, turbidity
    - Settlometer, Sludge judge
    - MLSS, MLVSS
    - Centrifuge spins
    - Microscopic evaluation
    - Oxygen Uptake Rate, Specific Oxygen Uptake Rate

Process Control

- Test performed in the aeration basin and or clarifier that indicate what the effluent quality will be when the water leaves the treatment plant.
- There may be a significant time delay from the time water enters the aeration basin and effluent sampling or discharge.
- Manufactures face similar quality challenge.

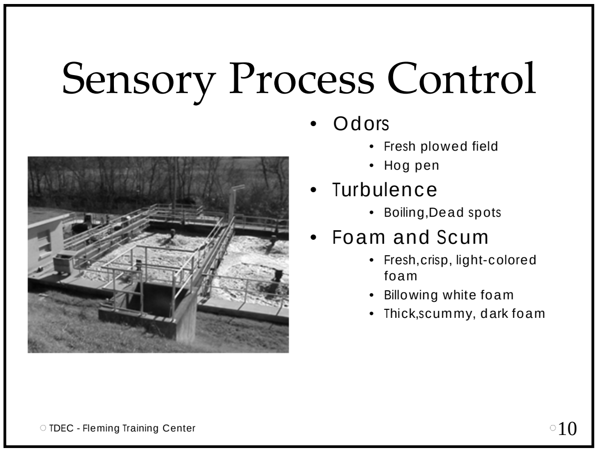


Process Control

What aerator test "now" will assure you of good effluent? When that water reaches the effluent several hours latter.

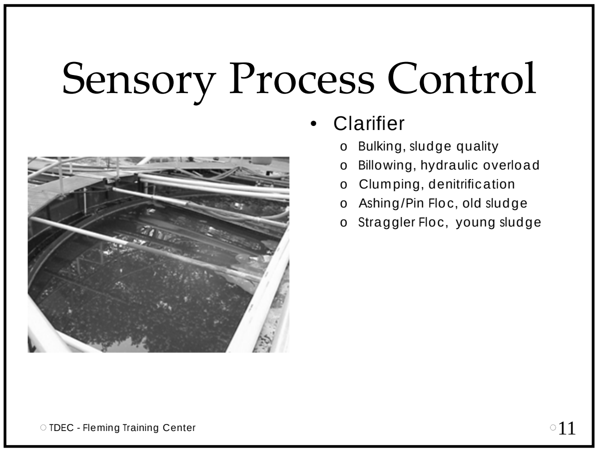
Process Control

- You choose the method that assures you that effluent will meet permit.
- NPDES permit
  - Part II.A.4 Proper Operations and Maintenance
    - "…adequate process controls…"
    - Though almost hidden, this is a Permit requirement
- Find a method that works for you and use it!



Sensory Process Control

- Odors
  - Fresh plowed field
  - Hog pen
- Turbulence
  - Boiling,Dead spots
- Foam and Scum
  - Fresh,crisp, light-colored foam
  - Billowing white foam
  - Thick,scummy, dark foam



Sensory Process Control

- Clarifier
  - Bulking, sludge quality
  - Billowing, hydraulic overload
  - Clumping, denitrification
  - Ashing/Pin Floc, old sludge
  - Straggler Floc, young sludge

Process Control

- Flow Rates, accurate flow measurements of premier importance.
- Locations
  - Influent Q
  - RAS,WAS, other
- Dissolved oxygen
  - >0.5mg/L for BOD removal, >2.0 for Ammonia
  - Profiles-longitudinal, vertical
  - DO levels are relative to the oxygen demand

Process Control, continued

- pH, 5-9 for BOD, 6.5-8 for Ammonia
  - o Indicator of toxicity
  - o Indicator of nitrification problems
- Temperature, Above freezing for BOD, 25°C optimal for AM.
  - o Use D.O. meter
  - o Effects speed of bacterial metabolism, or perhaps no metabolism!

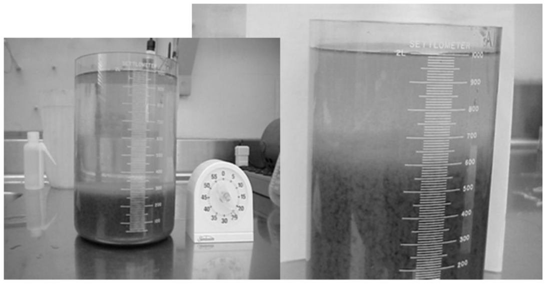
Process Control, continued

- Alkalinity, effluent >50mg/L for Ammonia removal
  - o Necessary for complete nitrification
- ORP-Oxidation Reduction Potential, Redox
  - o pH meter with ORP probe
  - o Indicated the oxidative state of the solution
- Turbidity
  - o Indicator of completeness of flocculation

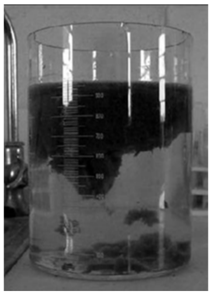
Process Control, continued

- Settleometer
  - o Use settlometer not graduated cylinder
  - o Indicator of clarifier performance
  - o How well the biomass- settles and compacts
- Sludge Judge, MLSS, MLVSS Centrifuge spins
  - o Indicators of biomass inventory

Settleometer

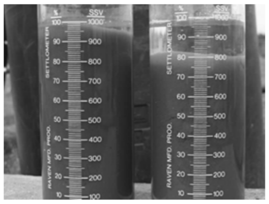


Settleometer



- Basic Process Control
- 5min, How fast sludge settles
- 30 min, How well sludge compacts
- Supernatant turbidity, how well sludge flocculates
- Denitrification

Clarifier Solids



- Activated Sludge Process Control:
  - o Mallory Cylinder or Settleometer
  - o Glass or plastic flat bottom beaker
  - o Sample (2 L) settles 30 min, then settled sludge volume read off side of cylinder
  - o During test, rate and quality of settling are observed

Sludge Volume Index (SVI)

- Monitor activated sludge settling behavior for process control
- Calculation using MLSS (mg/L) and 30 min SSV (mL/L)
- $$SVI = \frac{(mL/L\ after\ 30\ min)(1{,}000)}{MLSS\ mg/L}$$
- Target 100, Range 50-150

Settled Sludge Concentration (SSC)

- Centrifuge test for sludge quality
- Aeration tank mixed liquor concentration (ATC)
- Tubes contain 10 mL
- Centrifuge 15 min at 3000 rpm

Settled Sludge Concentration (SSC)

- Read level of compacted solids. Multiply result by 10 to obtain sludge concentration or % solids (ATC)
- Can be used on return and waste sludges
- Calculate SSC for any SSV as:
  - SSC = (1000)(ATC/SSV)
- Plot settleable vs. centrifuge solids for quick estimate of settleable solids

Biomass Inventory

- Inventory of Biomass should answer three questions
  - How much sludge is in the system?
  - Where is it located?
  - How long has it been there?
  - Experience has shown us certain sludge ages give us certain effluent qualities.
- With these answers, process control is easy

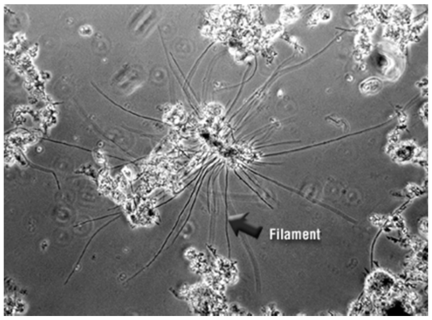
Biomass Inventory

- Thumb Rules
  - BOD Removal
    - MCRT, 0.5-1 Day
  - Ammonia Removal
    - MCRT 4-15 Days
- There are exceptions to all rules!!!

Microscopic Evaluation

- Floc analysis, Jenkins' Book
  - General shape, size, dispersed cells
- Protozoan/ Metazoan counts
  - General indicator of sludge age
- Filaments
  - Abundance, inside/outside flock, bridging
  - Non-Phase microscope, ID- Nocardia, Beggiatoa
- Slime Bulking
  - India ink test
-

Micrograph of Floc and Filaments

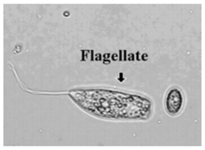


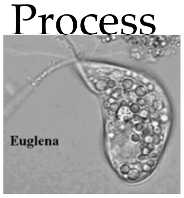
Protozoa

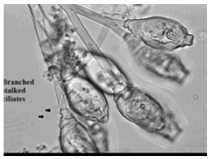
- Single-celled animals that also reproduce by binary fission
- Have complex digestive systems that ingest organic matter which they use as an energy and carbon source
- Graze on bacteria
- Form cysts
- Beneficial in wastewater treatment
- Indicators of health of system

- Examples:
  - Paramecium
  - Stalked Ciliates
  - Amoeba
  - Euglena
  - Flagellate

Protozoa Found in the Activated Sludge Process



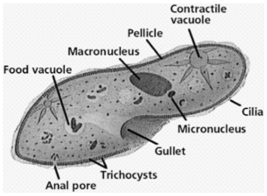




Protozoa

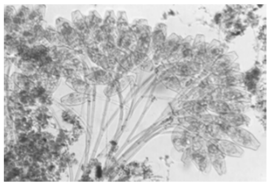
Free Swimming Ciliate - Paramecium

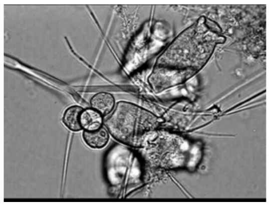
Paramecium eating



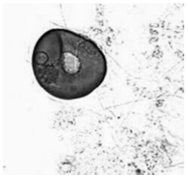
Protozoa

Stalked Ciliates

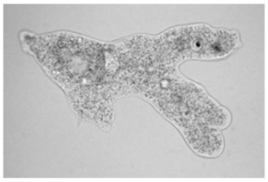




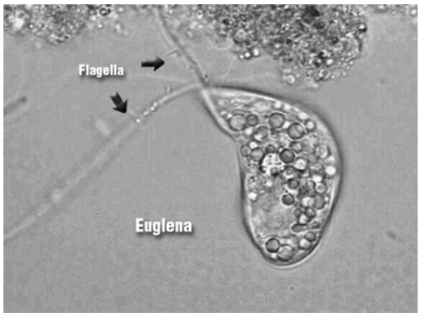
Protozoa



Amoeba eating



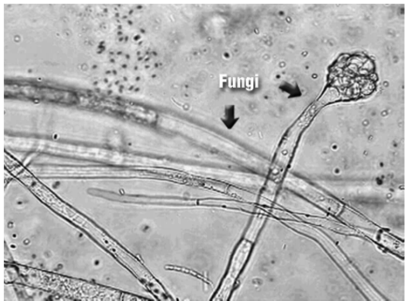
Protozoa



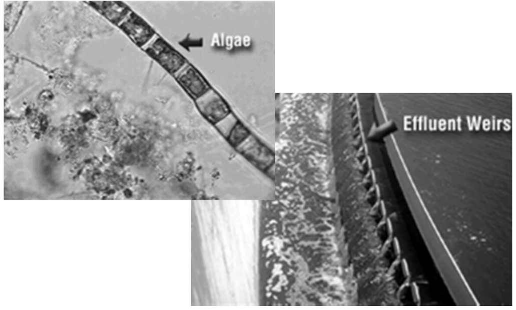
Fungi and Algae

- Algae
  - Photosynthetic
  - Eutrophication can cause algal blooms in receiving streams
  - Key in operation of wastewater ponds: produce oxygen needed by bacteria
  - Nuisance in clarifiers, basins, etc.
- Fungi
  - Soil organisms
  - Degrade dead organic matter (saprophytic)

Fungi



Algae



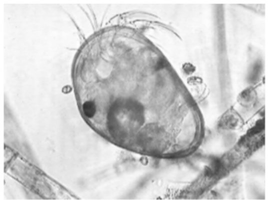
Worms


Ascaris and egg


Trickling filter

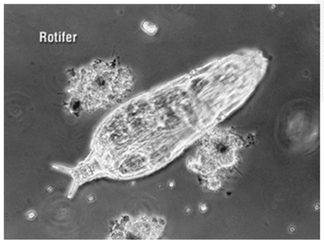
- Multicellular organisms
- Diseases (tapeworms, roundworms)
- Beneficial in trickling filters (increase air penetration in biofilm and help in sloughing)

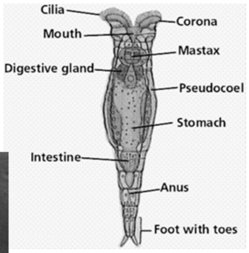
Metazoa



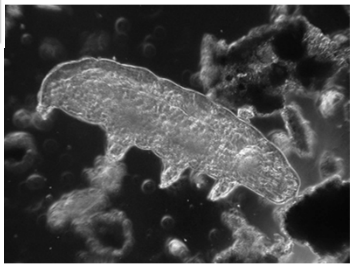
- Multi-cellular animals
- Examples:
  - Rotifer
  - Water Mite
  - Water Bear
  - Nematodes
  - Ostracods

Rotifer

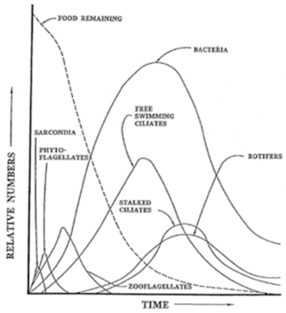




Water Bear



Bacteria Population vs. Sludge Age



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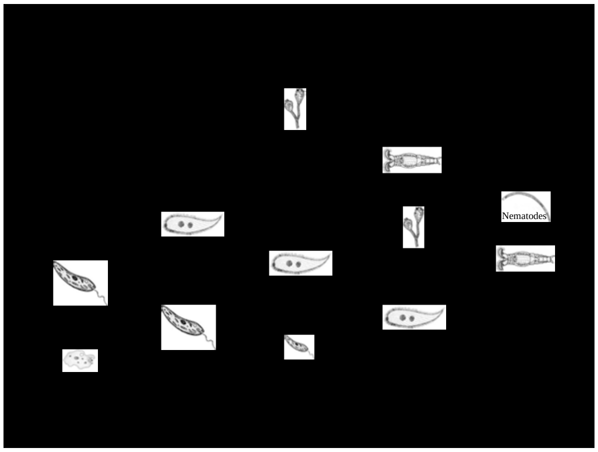


Microorganisms Predominance

- If conventional plant and you start to see more rotifers and less free-swimming ciliates, you need to increase wasting to make old sludge go away/
- If extended aeration plant and you have pin floc and nematodes, you are holding your sludge too long.

Oxygen Uptake Rate

- Rate at which microorganisms use oxygen
  - Indicator of speed of metabolism
  - Indicator of toxicity
  - Indicator of food abundance or ease of metabolism
- OUR varies with solids concentration
- SOUR accounts for solids variation
  - Commonly called Respiration Rate

SOUR Values

- SOUR >20mgO_2/hr/gm MLVSS
  - Logarithmic growth, Flagellates, dispersed flock
  - Settling Slow SSV_5>750cc/L
- SOUR 12-20mgO_2/hr/gm MLVSS
  - Declining growth, Ciliates, Flocks forming
  - Settling normal SSV_5=600-750 cc/L

SOUR Values

- Sour <12mgO2/hr/gm MLVSS
  - Endogenous Respiration, Rotifers and higher life
  - Pin Flock
  - Settling Fast, SSV_5<600cc/L

  - Remember the growth graph

Long Term Process Control

- F:M
- Food to Microorganism Ratio
- <u>lbs. of Raw BOD</u>

  lbs. of MLVSS
- Even bugs want a adequate diet.
- Always at least 5 days late.

Process Control

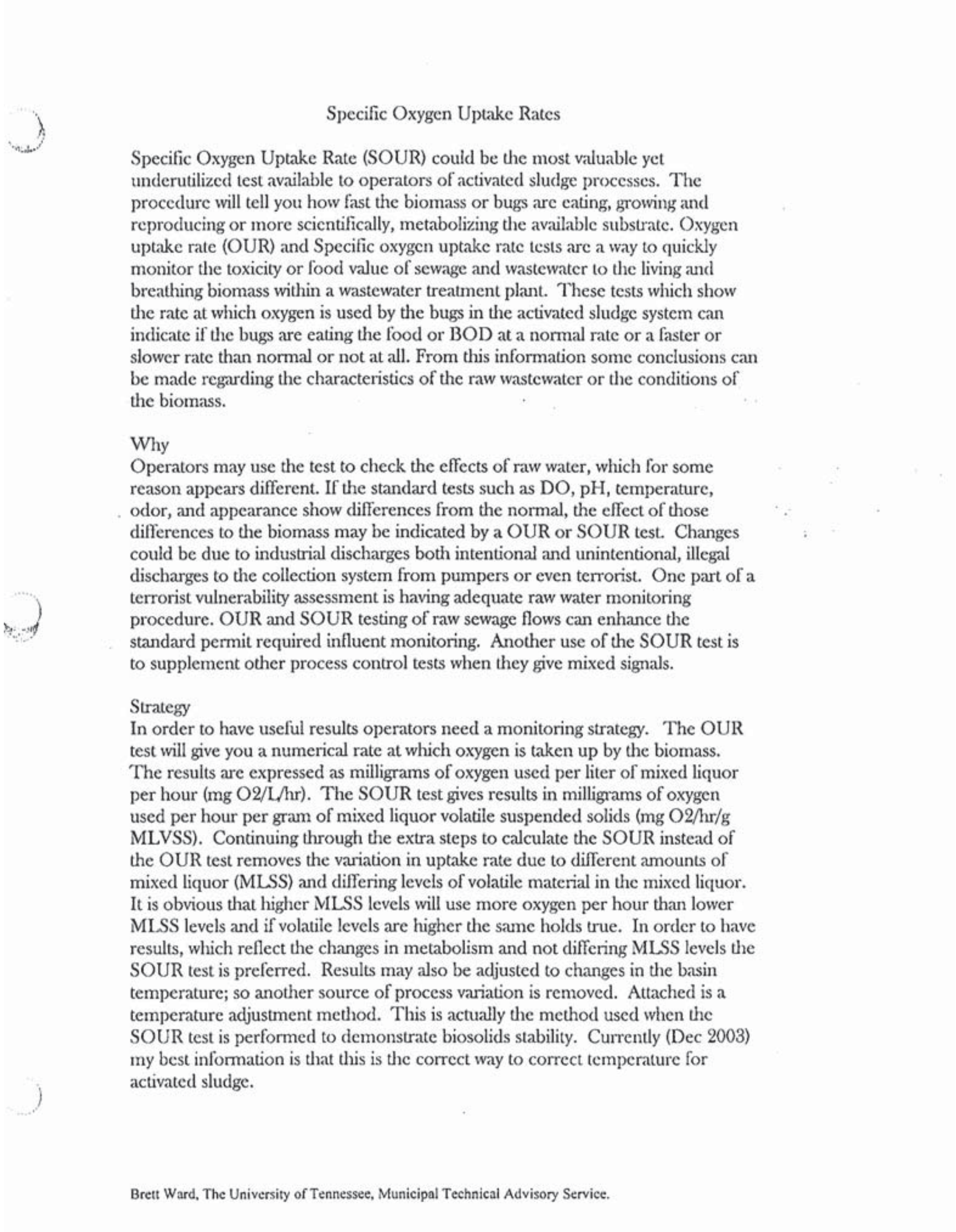
- Human senses
  - Visual appearance, odors
- Process tests
  - Flow, D.O., pH, temp., alkalinity,ORP, turbidity
  - Settlometer, Sludge judge
  - MLSS, MLVSS
  - Centrifuge spins
  - Microscopic evaluation
  - Oxygen Uptake Rate, Specific Oxygen Uptake Rate

Process Control

- Choose a method that works for you.
- Collect the Data.
  - Data is the voice of the process.
- Use the Data!
- Make decisions based on the data!
- Graph the Data!
  - Picture of the numbers, picture of the process.

Process Control

- Find a method that works for you
- Use it!
  - Meet your Permit
  - Do better than your Permit
  - Operate at a lower cost
  - Become a better operator



Specific Oxygen Uptake Rates

Specific Oxygen Uptake Rate (SOUR) could be the most valuable yet underutilized test available to operators of activated sludge processes. The procedure will tell you how fast the biomass or bugs are eating, growing and reproducing or more scientifically, metabolizing the available substrate. Oxygen uptake rate (OUR) and Specific oxygen uptake rate tests are a way to quickly monitor the toxicity or food value of sewage and wastewater to the living and breathing biomass within a wastewater treatment plant. These tests which show the rate at which oxygen is used by the bugs in the activated sludge system can indicate if the bugs are eating the food or BOD at a normal rate or a faster or slower rate than normal or not at all. From this information some conclusions can be made regarding the characteristics of the raw wastewater or the conditions of the biomass.

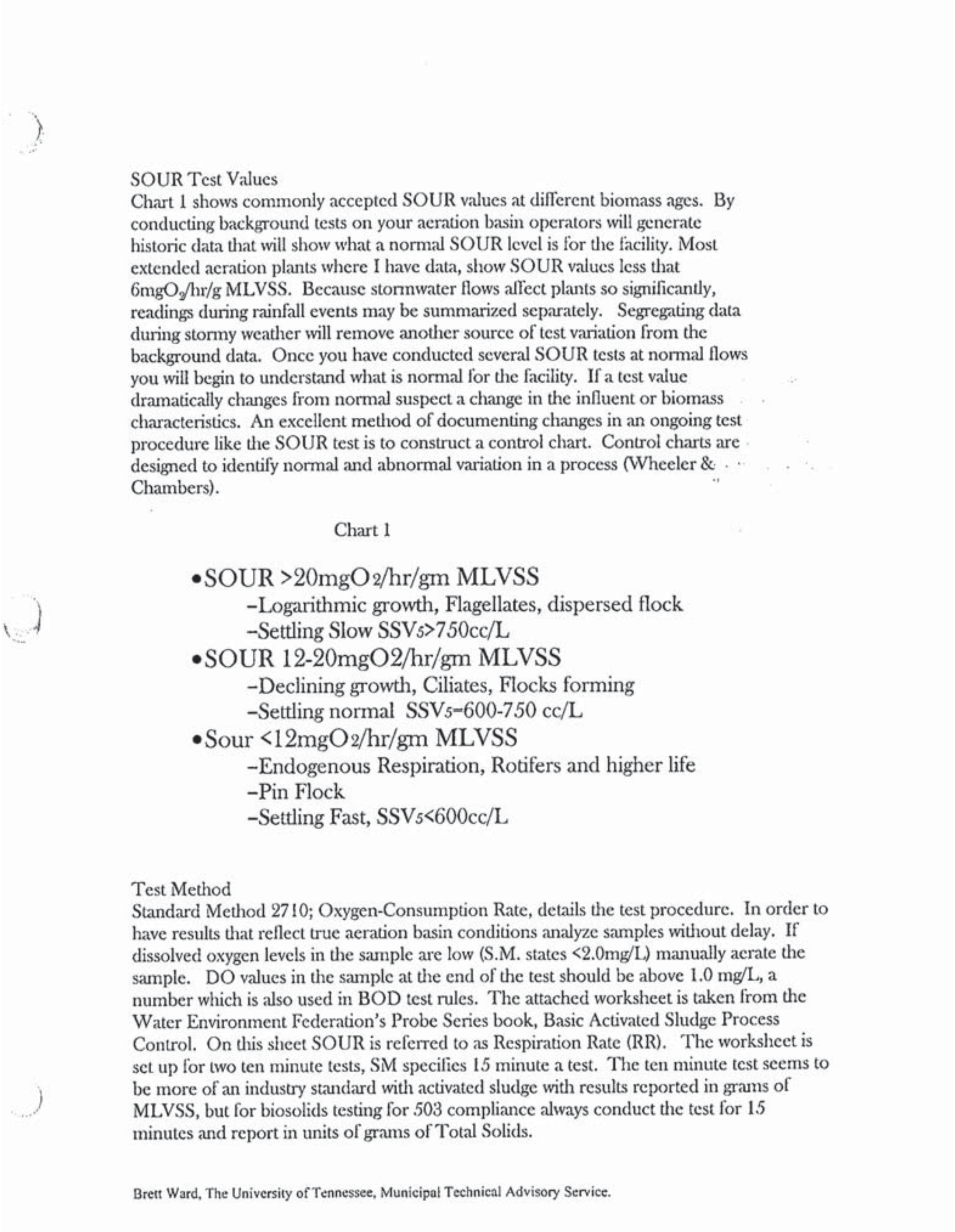
Why

Operators may use the test to check the effects of raw water, which for some reason appears different. If the standard tests such as DO, pH, temperature, odor, and appearance show differences from the normal, the effect of those differences to the biomass may be indicated by a OUR or SOUR test. Changes could be due to industrial discharges both intentional and unintentional, illegal discharges to the collection system from pumpers or even terrorist. One part of a terrorist vulnerability assessment is having adequate raw water monitoring procedure. OUR and SOUR testing of raw sewage flows can enhance the standard permit required influent monitoring. Another use of the SOUR test is to supplement other process control tests when they give mixed signals.

Strategy

In order to have useful results operators need a monitoring strategy. The OUR test will give you a numerical rate at which oxygen is taken up by the biomass. The results are expressed as milligrams of oxygen used per liter of mixed liquor per hour (mg O2/L/hr). The SOUR test gives results in milligrams of oxygen used per hour per gram of mixed liquor volatile suspended solids (mg O2/hr/g MLVSS). Continuing through the extra steps to calculate the SOUR instead of the OUR test removes the variation in uptake rate due to different amounts of mixed liquor (MLSS) and differing levels of volatile material in the mixed liquor. It is obvious that higher MLSS levels will use more oxygen per hour than lower MLSS levels and if volatile levels are higher the same holds true. In order to have results, which reflect the changes in metabolism and not differing MLSS levels the SOUR test is preferred. Results may also be adjusted to changes in the basin temperature; so another source of process variation is removed. Attached is a temperature adjustment method. This is actually the method used when the SOUR test is performed to demonstrate biosolids stability. Currently (Dec 2003) my best information is that this is the correct way to correct temperature for activated sludge.

Brett Ward, The University of Tennessee, Municipal Technical Advisory Service.



SOUR Test Values

Chart 1 shows commonly accepted SOUR values at different biomass ages. By conducting background tests on your aeration basin operators will generate historic data that will show what a normal SOUR level is for the facility. Most extended aeration plants where I have data, show SOUR values less that 6mgO$_2$/hr/g MLVSS. Because stormwater flows affect plants so significantly, readings during rainfall events may be summarized separately. Segregating data during stormy weather will remove another source of test variation from the background data. Once you have conducted several SOUR tests at normal flows you will begin to understand what is normal for the facility. If a test value dramatically changes from normal suspect a change in the influent or biomass characteristics. An excellent method of documenting changes in an ongoing test procedure like the SOUR test is to construct a control chart. Control charts are designed to identify normal and abnormal variation in a process (Wheeler & Chambers).

Chart 1

- SOUR >20mgO$_2$/hr/gm MLVSS
  - Logarithmic growth, Flagellates, dispersed flock
  - Settling Slow SSV_5>750cc/L
- SOUR 12-20mgO2/hr/gm MLVSS
  - Declining growth, Ciliates, Flocks forming
  - Settling normal SSV_5=600-750 cc/L
- Sour <12mgO$_2$/hr/gm MLVSS
  - Endogenous Respiration, Rotifers and higher life
  - Pin Flock
  - Settling Fast, SSV_5<600cc/L

Test Method

Standard Method 2710; Oxygen-Consumption Rate, details the test procedure. In order to have results that reflect true aeration basin conditions analyze samples without delay. If dissolved oxygen levels in the sample are low (S.M. states <2.0mg/L) manually aerate the sample. DO values in the sample at the end of the test should be above 1.0 mg/L, a number which is also used in BOD test rules. The attached worksheet is taken from the Water Environment Federation's Probe Series book, Basic Activated Sludge Process Control. On this sheet SOUR is referred to as Respiration Rate (RR). The worksheet is set up for two ten minute tests, SM specifies 15 minute a test. The ten minute test seems to be more of an industry standard with activated sludge with results reported in grams of MLVSS, but for biosolids testing for 503 compliance always conduct the test for 15 minutes and report in units of grams of Total Solids.

Brett Ward, The University of Tennessee, Municipal Technical Advisory Service.

Temperature Adjustment

SOUR is determined at the aeration basins ambient temperature and then adjusted as follows.

$$\text{SOUR@20°C} = \text{SOUR @ Ambient Temp.} * A^{(20\text{-Ambient temp.})}$$

Where A = 1.05 above 20°
= 1.07 below 20°
These factors are good between 10° C and 30° C

Simplified
SOUR @20° C = SOUR @ Ambient Temp. * Correction Factor

$$\text{Correction} = A^{(20\text{-Ambient Temp})}$$

Temp° C	Correction Factor
10	1.97
11	1.84
12	1.72
13	1.60
14	1.50
15	1.40
16	1.31
17	1.22
18	1.14
19	1.07
20	1.00
21	0.95
22	0.90
23	0.86
24	0.82
25	0.78
26	0.75
27	0.71
28	0.68
29	0.64
30	0.61

Brett Ward, The University of Tennessee, Municipal Technical Advisory Service.

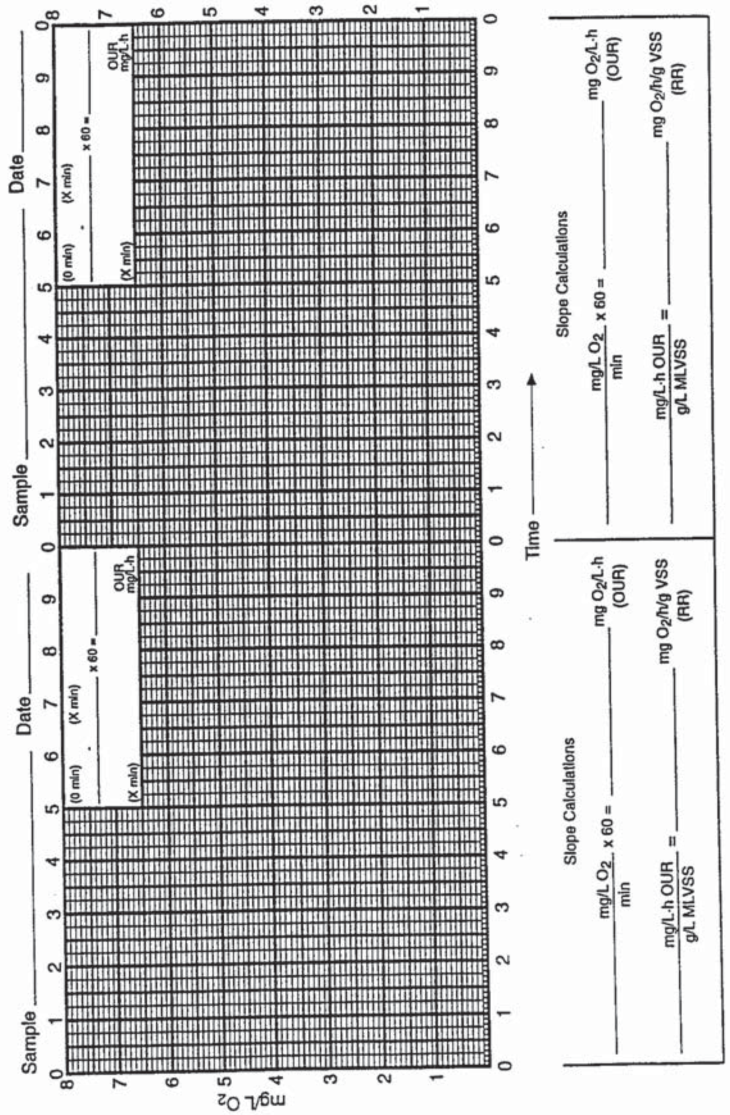
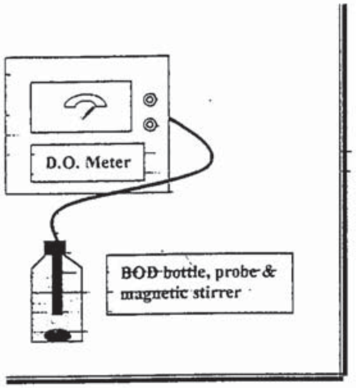


Figure 2.4 Respiration rate worksheet/graph.



OXYGEN UPTAKE TEST

I. Test Significance

O_2 uptake rate (or OUR) is the only quick test that an operator has at his disposal that allows him to assess feed acceptability as well as sludge quality. OUR is a simple test which can be run by an operator and it does not require elaborate lab skills..

II. Test Samples

- Mixed liquor (aeration tank effluent): This sample is the only sample referenced in Standard Methods, and consequently few individuals are familiar with the FED/UNFED OUR technique.
- Primary Effluent (FED mixture).
- Secondary Effluent (UNFED mixture): Unchlorinated.
- Return Sludge - Aerate as soon as possible after collection.

III. Test Frequency

- Once per day (during peak flow) to assess feed acceptability in large plants with industrial contribution. Perhaps even more frequently if plant has history of upsets.
- Once per week to assess sludge quality in small plants with no industry.

IV. Test Equipment

- Electronic DO analyzer w/bottle probe.
- Standard (300 ml.) BOD bottles.
- 250 ml. graduated cylinder.
- Magnetic mixer w/l to 1 ½" stirring bar.
- Sample containers.
- Tapered PVC (3/4" Pipe) Sleeve.
- Timer.
- Centrifuge.
- Centrifuge Tubes.

V. Test Procedure

Of course, OUR can be tested on the mixed liquor sample, but samples can be mixed together (Primary + Return = FED; Secondary + Return = UNFED) to assess activity after the return sludge is mixed with the incoming feed (FED). Additionally, an approximation of the oxygen used by the sludge after it is stabilized in the aeration basin and settled in the clarifier can be made (UNFED). Both samples are mixed such that the concentration approximates that of the mixed liquor.

Volume of sludge by flow measurements:

- *RSV = (AV – RSP)/(IFV – RSP).*

Where:

- RSV = Return Sludge Volume (ml.).
- AV = BOD Bottle Volume of 300 ml.
- IFV = Influent Flow Percentage of 100 % (decimal).
- RSP = Return Sludge Percentage of Influent Flow (decimal).

Note: Because of lags in the plant due to meter locations, tanks and detention times this technique has sometimes given erroneous results.

Volume of sludge by centrifuge:

- RSV = (ATC/RSC) x BOD Bottle Volume of 300 ml.

Where:

- ATC = Aeration Tank Concentration by Volume (%)
- RSC = Return Sludge Concentration by Volume (%)

Examples: RSP = 40% influent flow ATC = 3.0% RSC = 10.5%

- By flow: RSV = (300 x .40)/(1.0 + .40) = 86 ml.
- By centrifuge: RSV = (3.0/10.5) x 300 = 86 ml.

1. Calibrate DO meter.
2. Mix and pour RSV into BOD bottle.
3. Fill remainder of bottle (into neck to prevent air bubbles) with:
   - Primary effluent for FED sample.
   - Secondary effluent for UNFED sample.
4. Insert Tapered PVC Sleeve.
5. Place empty BOD bottle on top (ala Sludge Cocktail).
6. Aerate sample by transferring contents from one bottle to another and agitating.
7. Place back on magnetic mixer and remove bottle and PVC sleeve allowing any entrained air to escape.
8. Insert magnetic stirring bar and DO electrode assembly.
9. Adjust magnetic mixer to speed sufficient to maintain solids in suspension. Usually the mixer on the probe assembly is sufficient to suspend solids near the top of the bottle, but not always throughout the sample.

VI. Data Collection

- Prepare date record sheet including the following information (using centrifuge):

Date________Time________Temp.(°C)_____
RSC________ATC________RSV ________

	DO (mg/L.)	▲ DO mg./L./min.)
Minutes		
0 (start)		
1		
2		
3		
4		
5		
6		

Notice that RSP may be substituted for RSC and ATC if flows are used. Also, time intervals may require adjustment to half minutes for rapid OUR samples (e.g. FED). Adjust Time (min.) column to 0, 0.5, 1.0, 1.5, 2.0, etc.

- Observe and record initial temperature and DO, start the timer and observe and record the DO precisely at one-minute intervals. The DO meter should remain on during this phase so that the rate of change can be observed and half-minute readings can be taken if required.
- Record on data sheet until at least three (3) consistent ▲ DO readings are obtained.
- Record the final temperature. Caution should be taken that a hot-plate/magnetic mixer is not used with heat-on, as results will be extremely erroneous.
- Pour a sample for the test mixture into the centrifuge tube and test to check concentration. It should be very close to ATC.

VII. Special Variations or Applications of Test

Mixed liquor (Standard Methods)

Stabilization of the waste, as it leaves the aeration can be determined.

Stabilization Time Test.

1. Reflects sludge activity during the aeration cycle.
2. Indicates events in progression after feeding, e.g.
   - possible lags.
   - irregular progression or phases.
   - extended time requirements.
   - excessive peak OUR.
3. As stabilization time proceeds.
   - OUR decreases.
   - flocculation tendencies increase.
   - settling rate increases.
   - solids/liquid separation improves (within limits).
4. A curve depicting the stabilization process can be drawn from the data gathered in this simple test.

Procedure.

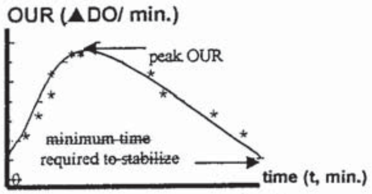
1. determine UNFED - OUR

2. mix and aerate the Return Sludge and Primary Effluent in a small tank (6 liters or more) in the same proportions as those that exist in the aeration basin using the ATC, RSC technique as before and knowing the final volume
3. (e.g. 6 L. = 20 BOD bottles).
4. upon initiation (t = 0) draw off 300 ml. bottle for OUR test.
5. perform OUR test and pour remainder of sample back into stabilization tank.

Data Collection.

- repeat OUR test every fifteen (15) minutes until OUR approaches UNFED - OUR.
- to develop the stabilization curve, plot OUR (△ DO/min) vs. time (min.).

Example - Stabilization Test Curve



- Permits operator to determine if:
1. adequate aeration time is available.
2. excessive oxygen demands are being placed on system (which might require a change in operating mode, e.g. place another available aerator into operation).
3. a new load is affecting system.

Normally, stabilization tests are developed only a few times a year of whenever the industrial contribution changes.

Aerobic Digesters (specific OUR or SOUR).

- SOUR expressed in mg. DO/gm. MLVSS/hr. may be used to determine the stability of aerobically digested sludges. Rates of 0.5 to 1.0 mg. DO/gm. MLVSS/hr. are normally associated with well-stabilized sludges.

BOD Estimates.

- An OUR test performed on undiluted (100%) unchlorinated secondary effluent can be correlated with BOD_5 data to obtain preliminary results on effluent quality. Although BOD is still required for reporting purposes, this can serve as an aid toward setting up dilutions for BOD measurement.
- ▲DO at 15-30 minute intervals gives an activity index related to effluent quality in less than four (4) hours.

VIII. Interpretation

Unfed Rate

UNFED follows the load during the day and week. For MLSS concentrations of 2500 - 3500 mg./L. the **UNFED should be approximately 0.3 - 0.7 mg. DO/L./min.**

To compare OUR on various sludges, the Specific Oxygen Uptake Rate (SOUR), a concentration independent value in mg. DO/hr./gm. can be determined:

- Calculate OUR in mg. DO/L./hr.: OUR = (mg. DO/L./min.) x (60 min./hr.) = mg. DO/L./hr.
- Divide by MLVSS to obtain SOUR: SOUR = (mg. DO/L./hr.) x (1000 mg./gm.) / (mg./L. MLVSS)

Example: OUR = 0.7 mg. DO/L./min. MLVSS = 2500 mg./L.

SOUR = 0.7 x 60 x 1000 / 2500 = 16.8 mg. DO/hr./gm. MLVSS 4.

Note: Optimum UNFED - OUR is about 12-20 mg. DO/hr./gm. MLVSS

Low UNFED <0.3 mg DO./L./min. or <0.5 mg. DO/hr./gm. MLVSS.

- Indicates starved, over-oxidized sludge (appropriate value for aerobic digester).
- Fast settling rates.
- Pin floc.
- Common to extended aeration systems - old sludge.
- Corrective Action - Increase wasting, decrease MLSS.

High UNFED >0.8 mg. DO/l/min. or >20 mg. DO/hr./gm. MLVSS.

- Indicates bulky, under-oxidized sludge (young).
- May go septic in clarifier.
- Poor settling and compactness.
- Corrective Action - Increase oxidation pressures.
  1. Contact or step-feed mode change may be required.
  2. Flocculation aid may be necessary to ~~achieve settling~~ and compactness.
  3. Monitor DO of return sludge and adjust to allow for maximum settleability w/o septicity.

Fed Rate

- FED follows availability and concentration of the feed. FED samples are more apt to change than UNFED. Measurement can provide time to anticipate likely changes and make corrections. **FED will likely range from two to five (2 - 5) times the UNFED (0.6-3.5 mg. DO/L./min.).**
- After feeding, OUR can be expected to increase relative to UNFED.
- Plant may impose limit on oxygenation capacity. Most systems should be able to handle FED of 2.0 - 2.5 mg./L./min. If not, or if FED higher, all or any below:
1. improved mixing may be necessary.
2. step-feeding to distribute load.
3. more or modification of aeration.
- Toxic loads will depress FED relative to UNFED:
1. ~~treatment will suffer.~~
2. coagulant or powdered activated carbon may help.
3. if repeated, identify problem along collection system.
4. if feed is suspect, check by using acceptable feed such as dextrose or sucrose to assess low activity.
   - if OUR increases, "bugs" are not assimilating food.
   - if OUR does not change, something in waste is toxic to "bugs".
5. a small increase can be due to dilute feed, poor quality feed, sick sludge or unfavorable conditions (e.g. too long clarifier sludge detention time).

Load Index (LF - load factor).

- Ratio of FED to UNFED: (FED/UNFED).
- Indicates activity before and after feeding.
- Good sludge and acceptable feed increases LF >1.0 due to no depression in FED.
1. LF <1.0 - inhibiting or toxic load.
2. LF >1.0 but <2.0 - dilute or stabilized load.
3. LF >2.0 but <5.0 - acceptable loading.
4. LF >5.0 - possible 02 supply problems.

Supplemental Data Sheet for Oxygen Uptake Test

OXYGEN UPTAKE RATE

DATE ______DAY ______MLVSS(mg./L)______ATC(%)______RSC(%)______

SAMPLE	grab	id______	fed	id______	unfed	id______
TIME (am/pm)	start	end	start	end	start	end
TEMP, T (°C)	start	end	start	end	start	end
TIME, t	DO	▲DO Depletion	DO	▲DO Depletion	DO	▲DO Depletion
0.0						
.5						
1.0						
.5						
2.0						
.5						
3.0						
.5						
4.0						
.5						
5.0						
.5						
6.0						
OUR, mg/L/hr						
$SOUR_T$, mg/gm/hr						
$SOUR_{20}$, mg/gm/hr						

[1] **Calculations: ▲DO,** Depletion = $D.O._0 - D.O._t$; **Determine slope** = $\text{Depletion}_t - \text{Depletion}_0 / \text{Time}_t - \text{Time}_0$

[2] **(Oxygen Uptake Rate) OUR** = slope with the time corrected to hours (multiply by 360 for time in seconds or by 60 for time in minutes).

[3] Specific Oxygen Uptake Rate at Given Temperature, $\textbf{SOUR}_T$ = OUR, mg/L/hr / MLVSS, gm/L

[4] Specific Oxygen Uptake Rate at 20° C, $\textbf{SOUR}_{20} = SOUR_T * (1.024)^{(T-20)}$

5

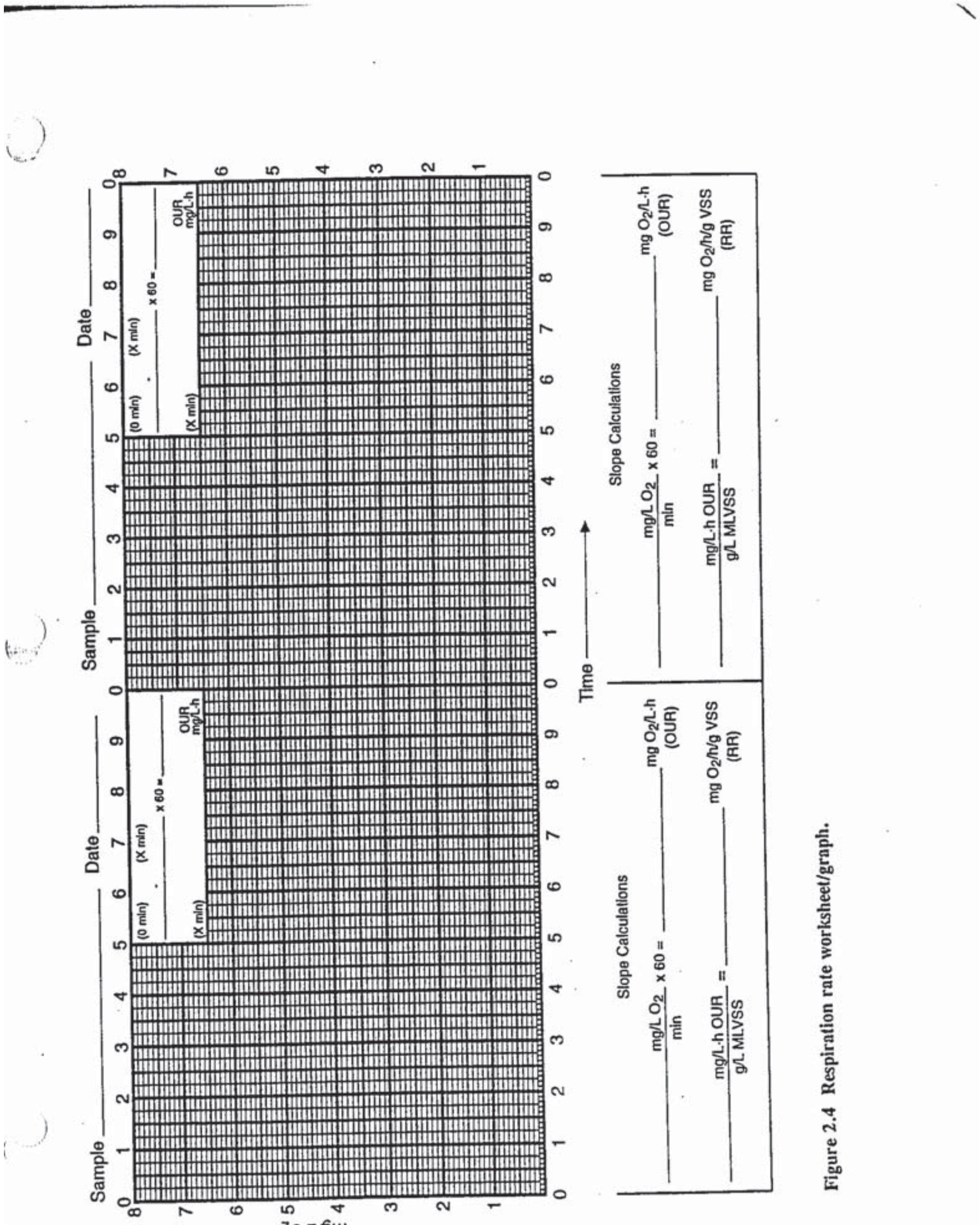


Figure 2.4 Respiration rate worksheet/graph.

The **oxygen uptake rate (OUR)** test is a quantitative method of measuring the rate at which oxygen is being utilized by all the organisms in the process. It is expressed as mg/L of oxygen used per unit of time such as mg O_2/min or mg O_2/hr. However, this number by itself is not very useful since it only suggests the rate at which oxygen should be added to the process. However, it can be used effectively to determine when the treatment process has been completed by performing a stabilization test. It can also be used in the fed and unfed test to determine the effect of the influent on the activated sludge.

The **fed** and **unfed** tests use the simple OUR test on simulated sludges. A "fake" activated sludge is developed to simulate conditions at the influent end of the aeration tank. It is a fake test because of the difficulty of collecting a truly mixed sample at that point. Another problem is keeping the sample from changing between sampling and analysis. The validity of this test relies on the changeable behavior of microorganisms. Since the results of these tests must be related to each other, the unfed test uses the same organisms for a "fake" test to simulate the conditions at the effluent end of the aeration tank. The unfed test indicates how active the microorganisms are at rest (endogenous respiration conditions), while the fed test shows the microorganism activity after feeding.

The fed test mixes influent with the correct amount of return sludge. The unfed test mixes secondary clarifier effluent (unchlorinated) with the correct amount of return sludge. Then the normal OUR test is done on each. The correct volume of return sludge in ml for each test is calculated by:

$$mL\ of\ return\ sludge = \frac{ATC}{RSC}\ x\ 300\,mlL$$

The 300 ml is the volume of a BOD bottle.

Fed/Unfed Ratios for Domestic Wastewater

<1	⇨	Toxic effect.
1-2	⇨	Dilute load or material hard to stabilize.
2-5	⇨	Normal.
>5	⇨	Very high organic load for the TSU available.

Once the two tests have been completed, a ratio of fed over unfed is calculated. If the ratio is less than 1.0, a toxic effect is shown since the fed would be using oxygen slower than the unfed. A value of 1-2 is found with very dilute influents or with wastes that contain slowly-degradable materials. A ratio between 2-5 is usually normal for a domestic waste while a value above 5.0 indicates extremely high loading. Industrial wastes can greatly affect this ratio and their effects must be evaluated on a plant-by-plant basis.

The **stabilization test** is a fed test but done with a large container such as an aquarium or a barrel. It is done to determine the amount of time required for the microorganisms to stabilize the waste. The fed mixture is aerated and samples are taken every hour. OURs are run on each sample to show the reduction in OUR as the microorganisms stabilize the waste. When the OUR becomes constant, the waste treatment process has been completed. If the actual stabilization time is greater than the detention time provided in the aeration tank, then process changes must be made. The total amount of sludge in the system could be increased to provide more microbes to stabilize the waste faster. Or, the return rate could be reduced to increase the actual detention time. Or, another aeration tank would have to be put on line. Or, finally, the organic loading reduced.

Since the OUR measures the oxygen used in the process per unit time, it does not indicate how fast each microorganism is using oxygen. To determine that rate, the oxygen uptake rate must be divided by the concentration of microorganisms in the system. The resulting number is the specific oxygen uptake rate (SOUR) or respiration rate. It indicates the biological activity of an activated sludge mixed liquor or an aerobically digesting sludge. The procedure and analysis for activated sludge mixed liquor is presented here, but the procedure to be used for the Part 503 regulation for sludge digestion systems is given in Standard Methods (APHA, 1992) as Method 2710 B, Oxygen-Consumption Rate.

The SOUR for a mixed liquor is the amount of oxygen in mg/L utilized by one gram of volatile suspended solids in the activated sludge in one hour or mg O_2/g VSS/hour. It suggests how fast each microorganism is using oxygen or how fast it is metabolizing food.

The SOUR provides more information than the OUR test in that the actual rate of microorganism activity is determined. All the OUR test does is determine how fast the mass of microorganisms is using oxygen. However, the operator already knows this information based on the amount of DO in the aeration tank. If the DO is low, the microorganisms are using the O_2 faster than it is supplied. If the DO is high, the O_2 is being supplied at a rate faster than the microorganisms use it.

OUR Test

Equipment

1. DO meter with BOD-bottle probe
2. 2-300 ml BOD bottles
3. Magnetic stirrer
4. Sample
5. Timer
6. Beaker with diameter slightly larger than BOD bottle

Procedure

1. Measure and record DO and temperature at the sample site.
2. Collect 3-4 liters of sample. The same sample can be used for suspended solids, settleability, centrifuge spins, microscopic exam, etc.
3. Pour some mixed liquor inside the beaker so that when the BOD bottle is placed in the beaker, the mixed liquor will come up to the neck of the bottle. This provides a more constant temperature in the BOD bottle during the test.
4. Thoroughly mix the sample, remove an aliquot and aerate it to get the DO above 5 mg/L. There are several methods that have been used to aerate the sample.
   a. Fill a 300-ml BOD bottle with sample. Mix and aerate the sample by placing a PVC adapter between the filled bottle and an empty BOD bottle. Pour the sample back and forth to aerate.
   b. Fill a one-liter container half full with mixed sample. Cap the container and gently shake. Fill the BOD bottle and begin.
5. Place the BOD bottle into the beaker. Be sure that the mixed liquor level in the beaker remains just below the neck of the BOD bottle.
6. Place the DO probe in the bottle, making sure not to trap any bubbles. Begin stirring the sample. Allow a short time for the temperature and the probe to stabilize.
7. Record the DO every minute for 10 minutes, or until the DO drop becomes consistent, or until the DO drops below 1 mg/L. Make sure that there is at least 1 mg/L difference between the start and finish of the test.

Note: If the OUR is extremely high, values may have to be read every 30 seconds and the initial DO may have to be increased to 7-8 mg/L. If that does not provide a satisfactory test, dilute the sample with BOD dilution water, reaerate the sample, and complete the test again. Calculate the appropriate dilution factor to obtain the OUR of the original sample.

OUR Calculations

1. Graph the "line of best fit" of the data. The line should be nearly straight. If there are data at the beginning and/or end of the test that are significantly different from the straight line, disregard those data. The straight line represents the actual microorganism activity.

2. Determine the slope of the line. Pick the points at the beginning and end of the straight-line portion. Divide the change in DO between those two points by the change in minutes between those two points.

3. OUR = ΔDO/ΔTime = mg O_2/L/min if the time is in minutes

   Multiply by 60 min/hr to get the results in mg O_2/L/hr

SOUR Calculations and Interpretation

Required information:
OUR of the sample
MLSS or MLVSS of the same sample

Calculation

$$SOUR = \frac{OUR,\ mg/L/hr \times 1{,}000\ mg/gm}{MLVSS,\ mg/L}$$

Interpretation

Conventional activated sludge normal range	- 12-20 mg O_2/hr/gm
Extended aeration normal range	- 6-12 mg/O_2/hr/gm

Values outside these ranges are not necessarily a problem since your plant may work well. However, they would normally mean that one should begin looking for potential problems.

Oxidation Reduction Potential Use in Wastewater Treatment

by

Brett Ward
Utility Operations Consultant
Municipal Technical Advisory Service
University of Tennessee

Oxidation Reduction Potential is an old test parameter that is seeing renewed popularity in wastewater applications. Readings give wastewater operators better insight into what wastewater microorganisms are actually doing in a particular situation or in a specific basin. This measurement parameter, which predates the dissolved oxygen meter, is useful in monitoring all types of biochemical reactions and is gaining popularity in monitoring wastewater processes which occur at dissolved oxygen levels below 1.0 mg/L. ORP readings also supplement D.O. readings by adding additional dimension of oxidative state to the positive D.O. readings. This ability to monitor and control anoxic and anaerobic respiration in a system can improve effluent quality, assist in odor control, and is key in effective biological nutrient removal. In the aeration basin ORP is proving to be effective in reducing operating cost while maintaining high effluent quality. ORP is also being used to monitor and control chlorination and dechlorination.

Oxidation Reduction Potential, also known as ORP, Redox, Redox potential, or electrical potential, is measured using the mV scale of a pH meter which is equipped with an ORP probe. The reading is a positive or negative number. The readings will tell an operator what the oxidizing state of the solution is at that time. Positive numbers indicate the presence of oxidizers or electron acceptors like oxygen and nitrate. Negative numbers indicate the presence of reduced compounds such as BOD and NH_3 which need oxidizing in order for stabilization to take place. In the table (Gronoszy) shows different ORP readings, the electron acceptor and the type of conditions that exist at those mV levels. When aerobic or fully oxic conditions exist, oxygen is needed as the reduced compounds are oxidized. In these conditions BOD removal occurs, ammonia is oxidized into nitrate and polyphosphate development occurs. In anoxic conditions the facultative organisms use nitrate oxygen as an oxygen source and denitrification occurs. Polyphosphate breakdown occurs as the ORP declines below zero. At ORP readings of negative 50mV and below, anaerobic conditions exist. Here respiration creates odors, organic acids and methane.

Aerobic Processes

ORP levels of positive 50mV and above are in the oxic range, and aerobic respiration is taking place with oxygen being the final electron acceptor. Though a complex biochemical pathway, the net effect is that the bacteria oxidize the highly reduced or high energy organic matter in the waste stream transferring electrons to oxygen and producing CO_2, and H_2O and

energy that the bacteria use for maintenance, growth and reproduction. This process is basically the reverse of photosynthesis. Other aerobic processes are nitrification where ammonia is oxidized into nitrite and then nitrate. And polyphosphate is formed by the bacteria removing phosphorus from the water.

Anoxic Processes

At oxygen readings of 0.0 mgL the bacteria must seek other sources of oxygen. The most easily available oxygen is that which is attached to nitrogen atoms forming nitrate (NO_3). When nitrate is available, the bacteria will denitrify the wastewater using the nitrate oxygen as an electron acceptor and releasing the nitrogen atom as nitrogen gas. If this occurs in the secondary clarifier, sludge clumping can be a problem and there can be solids washout; but if it occurs in the aeration basin at the operator's direction, the clarifier problems are avoided and the water has undergone biological nitrogen removal.

Anaerobic Processes

In order to biologically remove phosphate from wastewater there must be an anaerobic cycle where the polyphosphate bacteria use their stored polyphosphate energy and feed on the organic acids of the fermenting wastewater. This occurs at ORP readings of -50 to -200mV. In the wastewater collection system the formation of hydrogen sulfide occurs as the bacteria reduce sulfate in the water in their search for an electron acceptor as they oxidize fermentation products in the waste stream. As the electrical potential readings continue to decline, the acid forming and methane forming organisms begin to predominate the environment.

Instruments

Standard pH meters are used to measure ORP. The millivolt (mV) scale is used along with an ORP probe. Portable equipment can be purchased for less than $800. The most common probe currently in use uses a silver/silver chloride reference electrode (Ag/AgCl). All readings included in this paper are or have been converted to Ag/AgCl values. Other reference electrodes are the Calome and Standard Hydrogen. When comparing ORP readings, you must know which reference electrode is being used. Standard Methods defines the electrical potentials (E) for the three electrodes as follows:

$$\text{ORP } (E_C) + 45\text{mV} = \text{ORP } (E_{Ag/AgCl}) + 210 = \text{ORP } (E_H), \text{ (Std.Methods)}$$

There is no calibration of the meter when reading ORP, but the probe should be checked for accuracy using the available standards.

Standards, milli Volts readings for the $E_{Ag/AgCl}$ electrode
Light's Solution = +475mV
ZoBell's Solution = +228mV

pH 4 and Quinhydrone = +263 mV
pH 7 and Quinhydrone = +86 mV
mV change from pH 4 to 7 should be 177mV ± 20mV

If readings do not match the standards, the probe may need to be cleaned. Initial cleaning of a new probe should include liquid detergent, warm water and a soft brush followed by rinsing with DI water. Inorganic scale can be removed by soaking the probe for a few minutes in 5% Hydrochloric Acid. The platinum band may be polished with toothpaste. To remove oil and grease use acetone followed with detergent and rinsing (Broadley James). If the probe remains incorrect after cleaning, it should be discarded.

ORP Uses

ORP can be used to monitor and control a variety of wastewater processes and for spot checking of treatment plants and collection systems. Online controls of disinfection, aeration, sludge digestion and oxidant feed are all possible. Spot checking of wastewater systems using ORP can also be helpful in process control and troubleshooting.

Collection System

In the collection system odors begin to be produced at a reading of -50mV (Goronszy) though Mosey disputes this level saying the odors are not produced until -450mV. The difference may be the location of the measurement. Mosey's is a calculation based upon what the bacteria are doing in the slime layer, while Goronszy's appears to be a reading in the water above the slime. ORP measures can help operators pinpoint where in the collection system odor generation is most severe. They can also be used to monitor the dosage of various odor control oxidizers.

Aeration Basin

ORP is linearly related to the log of the dissolved oxygen. Goronszy reports that polyphosphate development begins and BOD removal begins at a reading of +50mV and ammonia removal at +100mV. Denitrification occurs in the anoxic zone or +50 to -50mV. Simultaneous nitrification and denitrification have been reported at +125mV by Muriyama. The theory is that outside the flock particle conditions are oxic but inside the flock particle there is a small anoxic zone where denitrification takes place.

When biological nutrient removal is being performed, ORP measurements give operators a definite on and off point for aeration control that incorporates not only the oxic spectrum where the D.O. meter can read, but also the anoxic and anaerobic spectrum where the D.O. meter cannot read. In situations where continuous ORP monitoring is performed on cycle aerated aeration basins a strip recorder will show definite inflection points on the plot of mV. As D.O. and ORP rise and fall, the rate of change in the mV reading has definite inflections at the points where nitrification or denitrification has been completed. It is at these points that aeration is changed to suit the exact process needs. The importance of knowing when a certain process is completed is to prevent excess aeration and its added cost or insufficient aeration and its potential problems. Charpentier reports two activated sludge plants with 20% power savings using cycle aeration controlled by ORP.

Sludge Digestion

ORP can be used to control both aerobic and anaerobic sludge digesters. In the aerobic digester benefits of cycle aeration controlled by ORP or pH (Al-Ghusain) include alkalinity recovery, energy savings (to 43%, Yu) volatile solids reduction, fecal reduction and improved dewaterability (Peddie). As breakdown of solids occurs, expect longer cycles to be needed to reach desired ORP levels.

Molof reports that the optimum ORP for anaerobic digestion is - 400 to -500mV. He further recommends that probes be installed within the digester because of the slow response of the probes to these very low readings and the difficulty of making accurate readings in material withdrawn from the digester.

Disinfection

Several chlorinator manufacturers are using ORP instead of chlorine residual to control the feed of chlorine into contact chambers and the feed of dechlorinating materials. In a disinfection situation, ORP would be reading the combined oxidizing power of chlorine and oxygen in the water. Disinfection set points should be in the +500 to +550mV range (Spriggs). Kim reports a fecal coliform level of 2.2 MPN at an ORP of +520mV. The World Health Organization has set a drinking water standard at +650mV and +750mV is the European Pool and Spa standard. Dechlorination is made to a ORP reading of +200mV(Spriggs, Kim). An advantage of ORP control over chlorine residual is that as pH decreases and the killing power of chlorine ions increases, ORP also increases.

Summary

Oxidation Reduction Potential is another tool available to wastewater personnel. It's a way to better understand what the microorganisms are doing. And if we know what they are doing, we can predict what the results will be. For the operator or engineer who is working toward optimizing plant performance and economy, ORP values can be very helpful. By using online ORP measurements operators or computer control equipment can better manage the aeration process for nutrient removal. There can be significant power savings by cycling the aeration using ORP readings to determine the best off and on levels to prevent over aeration or under aeration. In the collection system, ORP readings can be used to locate odor producing thresholds in the system and to monitor oxidizer feed dosages used to prevent those odors. ORP can also be used to monitor both the aerobic and anaerobic digesters as well as the chlorination

SETTLEABILITY BENCH SHEET

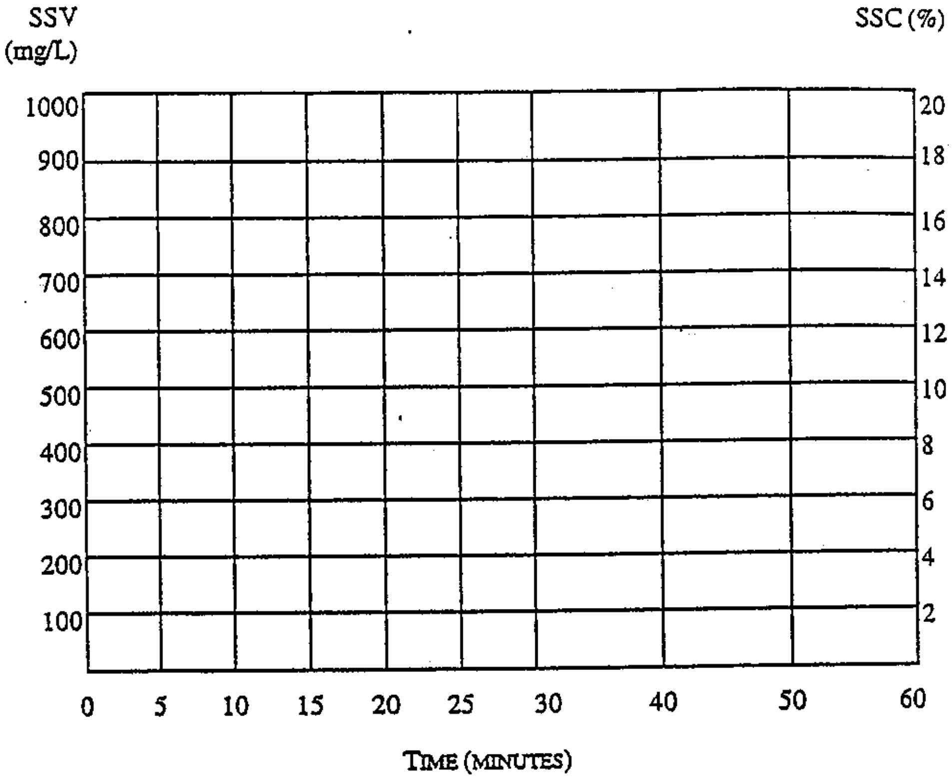
DATE_______________

TIME_______________

ANALYST_______________

TIME	SSV ML/L	SSC %
0		
5		
10		
15		
20		
25		
30		
40		
50		
60		

Aeration Tank Concentration (ATC) = _________________________ %



$$SSC = \frac{1000\ ATC}{SSV}$$

Observations:

Floc
- ☐ flocculant
- ☐ dispersed

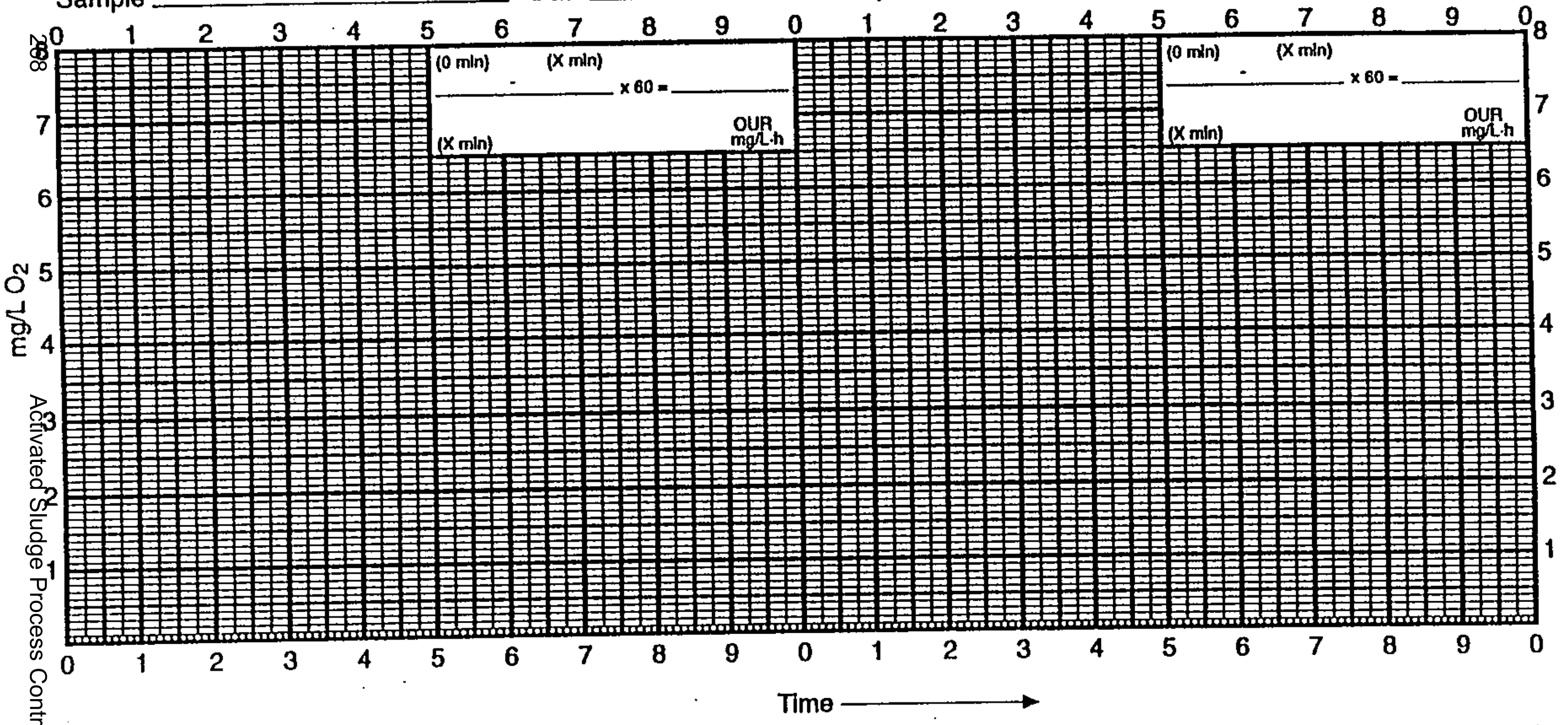
Interface
- ☐ well defined
- ☐ ragged

Supernatant
- ☐ clear
- ☐ turbid
  - ☐ pin floc
  - ☐ straggler floc

Comments: (order, color, etc.)

Rise Time____________hrs

Sample ______________ Date ______________ Sample ______________ Date ______________



Slope Calculations

______ $\frac{\text{mg/L } O_2}{\text{min}}$ x 60 = ______ mg O_2/L·h (OUR)

______ $\frac{\text{mg/L·h OUR}}{\text{g/L MLVSS}}$ = ______ mg O_2/h/g VSS (RR)

Slope Calculations

______ $\frac{\text{mg/L } O_2}{\text{min}}$ x 60 = ______ mg O_2/L·h (OUR)

______ $\frac{\text{mg/L·h OUR}}{\text{g/L MLVSS}}$ = ______ mg O_2/h/g VSS (RR)

Figure 2.4 Respiration rate worksheet/graph.

Section 17
Nutrients

Nutrients

Nitrate-Nitrite, Ammonia
Total Kjeldahl Nitrogen,
Phosphorus

Nitrogen Group - N

- In water and wastewater the forms of nitrogen that are of greatest interest are:
  - Nitrate
  - Nitrite
  - Ammonia
  - Organic Nitrogen

$\left.\begin{array}{l}\text{Nitrate}\\ \text{Nitrite}\\ \text{Ammonia}\end{array}\right\}$ Inorganic Nitrogen

$\left.\begin{array}{l}\text{Nitrate}\\ \text{Nitrite}\\ \text{Ammonia}\\ \text{Organic Nitrogen}\end{array}\right\}$ Total Nitrogen

Phosphorus Group - P

- Phosphorus occurs in natural water and wastewater almost solely as phosphates
- Types of Phosphorus Analyses include:
  - Total Phosphorus
  - Ortho-Phosphorus
  - Dissolved Phosphorus

Containers, Preservation & Holding Times

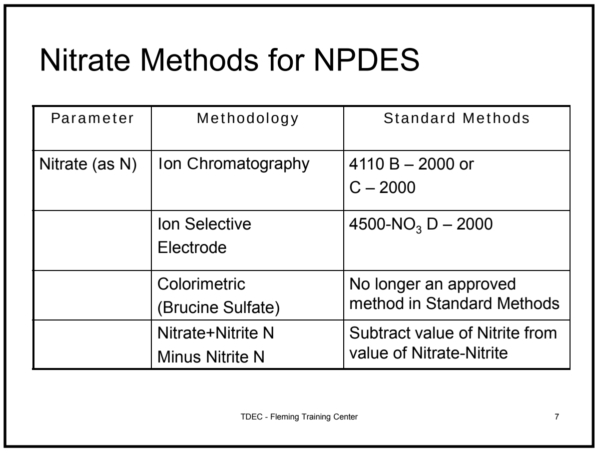
Parameter	Container	Preservation	Max. Holding
Nitrate	P, G	Cool, ≤ 6° C	48 hours
Nitrite	P, G	Cool, ≤ 6° C	48 hours
Ortho-phosphate	P, G	Cool, ≤ 6° C	48 hours
Nitrate + Nitrite	P, G	Cool, ≤ 6° C, H_2SO_4 to pH <2	28 days
Ammonia	P, G	Cool, ≤ 6° C, H_2SO_4 to pH <2	28 days
Kjeldahl Nitrogen,	P, G	Cool, ≤ 6° C, H_2SO_4 to pH <2	28 days
Phosphorus, Total	P, G	Cool, ≤ 6° C, H_2SO_4 to pH <2	28 days

NO_3+NO_2 Methods for NPDES

Parameter	Methodology	Standard Methods
NO_3+NO_2-N	Ion Chromatography	4110 B – 2000 or C – 2000
	Automated Hydrazine	4500-NO_3 H – 2000
	Cd-reduction, automated	4500-NO_3 F – 2000
	Cd-reduction, manual	4500-NO_3 E – 2000

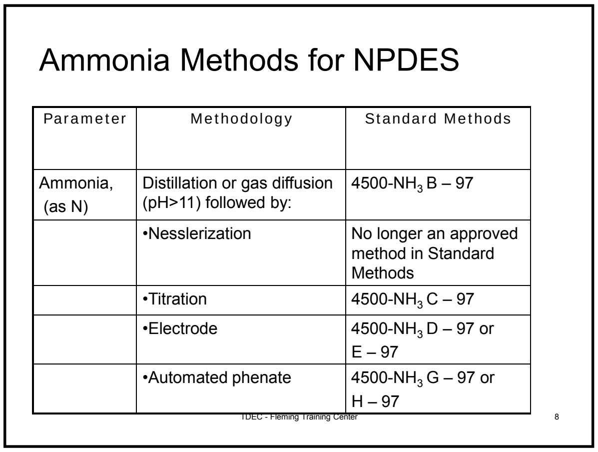
Nitrite Methods for NPDES

Parameter	Methodology	Standard Methods
Nitrite (as N)	Ion Chromatography	4110 B – 2000 or C – 2000
	Spectrophotometric (Manual)	4500-NO_2 B – 2000
	Automated, by pass Cd-reduction column	4500-NO_3 F – 2000



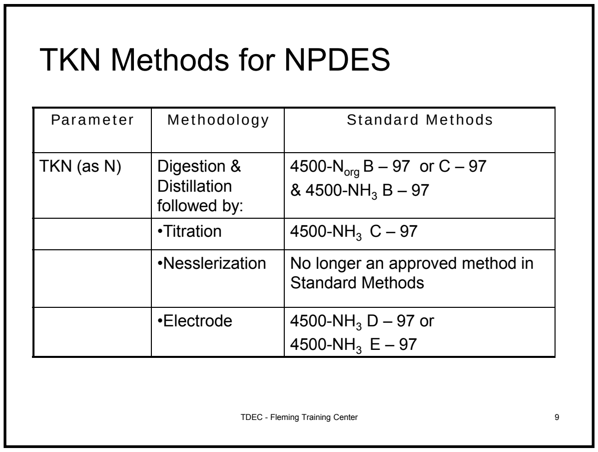
Nitrate Methods for NPDES

Parameter	Methodology	Standard Methods
Nitrate (as N)	Ion Chromatography	4110 B – 2000 or C – 2000
	Ion Selective Electrode	4500-NO_3 D – 2000
	Colorimetric (Brucine Sulfate)	No longer an approved method in Standard Methods
	Nitrate+Nitrite N Minus Nitrite N	Subtract value of Nitrite from value of Nitrate-Nitrite



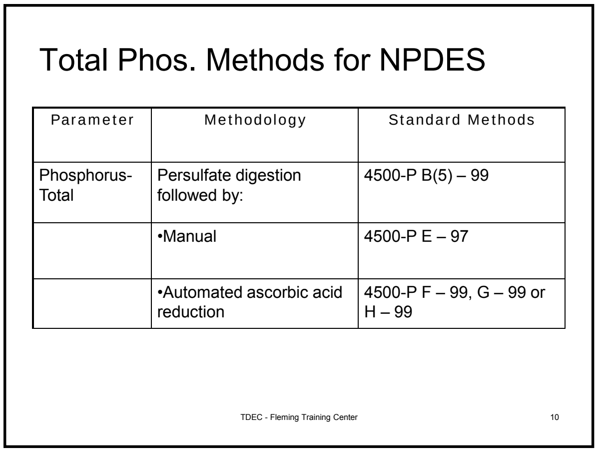
Ammonia Methods for NPDES

Parameter	Methodology	Standard Methods
Ammonia, (as N)	Distillation or gas diffusion (pH>11) followed by:	4500-NH_3 B – 97
	•Nesslerization	No longer an approved method in Standard Methods
	•Titration	4500-NH_3 C – 97
	•Electrode	4500-NH_3 D – 97 or E – 97
	•Automated phenate	4500-NH_3 G – 97 or H – 97



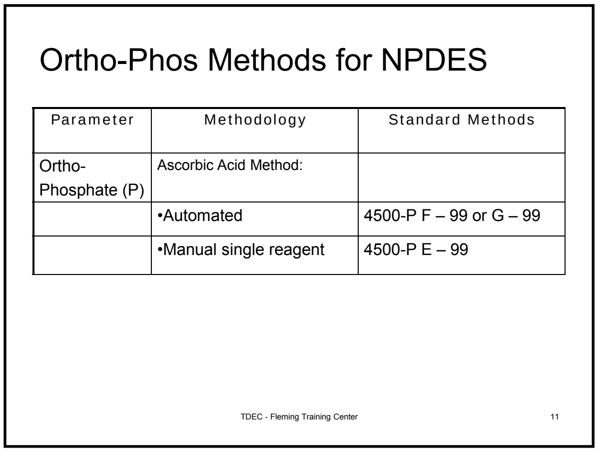
TKN Methods for NPDES

Parameter	Methodology	Standard Methods
TKN (as N)	Digestion & Distillation followed by:	4500-N_{org} B – 97 or C – 97 & 4500-NH_3 B – 97
	•Titration	4500-NH_3 C – 97
	•Nesslerization	No longer an approved method in Standard Methods
	•Electrode	4500-NH_3 D – 97 or 4500-NH_3 E – 97



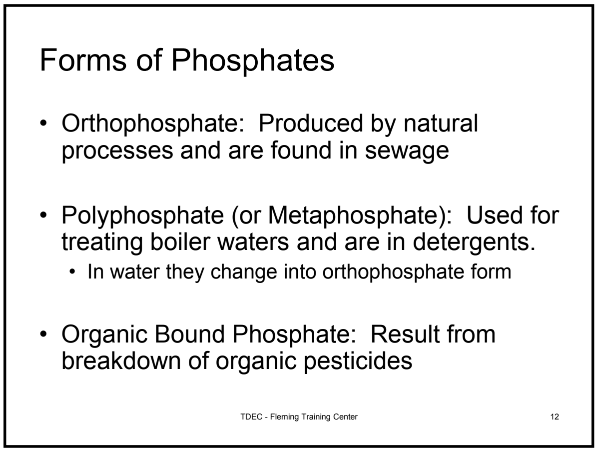
Total Phos. Methods for NPDES

Parameter	Methodology	Standard Methods
Phosphorus-Total	Persulfate digestion followed by:	4500-P B(5) – 99
	•Manual	4500-P E – 97
	•Automated ascorbic acid reduction	4500-P F – 99, G – 99 or H – 99



Ortho-Phos Methods for NPDES

Parameter	Methodology	Standard Methods
Ortho-Phosphate (P)	Ascorbic Acid Method:	
	•Automated	4500-P F – 99 or G – 99
	•Manual single reagent	4500-P E – 99



Forms of Phosphates

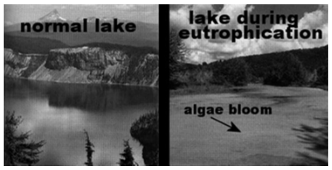
- Orthophosphate: Produced by natural processes and are found in sewage
- Polyphosphate (or Metaphosphate): Used for treating boiler waters and are in detergents.
  - In water they change into orthophosphate form
- Organic Bound Phosphate: Result from breakdown of organic pesticides

Environmental Impact

- Phosphate will stimulate the growth of aquatic plants
- High levels of phosphates entering waterways can stimulate algae and water plants to grow wildly which chokes up the waterways and uses up large amounts of dissolved oxygen

Algal growth fueled by nutrients running off agricultural fields and from urban areas) spreading into the Gulf of Mexico off the coast of Florida.

Environmental Impact



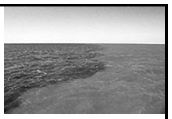
- Eutrophication or over-fertilization of receiving water from wastewater effluents
- Digestive problems can occur from extremely high levels of phosphate

Eutrophication

- Eutrophication is an increase in chemical nutrients (compounds containing nitrogen or phosphorus) in an ecosystem, and may occur on land or in water.
- However, the term is often used to mean the resultant increase in the ecosystem's primary productivity (excessive plant growth and decay), and further effects including lack of oxygen and severe reductions in water quality, fish, and other animal populations.

- Once algae blooms, it will die off and as the algae decay bacteria will consume it and use up all the oxygen.

Eutrophication



- Gulf of Mexico
  - Currently the most notorious dead zone is a 8,543 mi² region in the Gulf of Mexico, where the Mississippi River dumps high-nutrient runoff from its vast drainage basin, which includes the heart of U.S. agribusiness, the Midwest.
  - The drainage of these nutrients are affecting important shrimp fishing grounds.
  - This is equivalent to a dead zone the size of New Jersey.

Eutrophication Video

Reversal of Dead Zones

- Dead zones are reversible.
- The Black Sea dead zone, previously the largest dead zone in the world, largely disappeared between 1991 and 2001 after fertilizers became too costly to use following the collapse of the Soviet Union and the demise of centrally planned economies in Eastern and Central Europe.
- Fishing has again become a major economic activity in the region

Most Common Procedures

- Orthophosphate is the amount of inorganic phosphorus in a sample and is measured by direct colorimetric procedures
- Total phosphorus is the amount of all phosphorus present in the sample regardless of form and is measured by the persulfate digestion procedure followed by the colorimetric analysis

Common Findings in Testing

Common Ranges	Influent	Effluent
Total Phosphorous	4 – 12 mg/L	2 – 10 mg/L
Orthophosphate	2 – 8 mg/L	1 – 6 mg/L

Acid Persulfate Digestion Method EPA Approved

- Gather sample
- Measure 50 ml into Erlenmeyer flask
- Add .5 grams of Potassium Persulfate and mix
- Add 2.0 ml of 5.25 Normality Sulfuric Acid Solution
- Place flask on hot plate and Boil gently for 30 minutes
- Cool sample to room temperature
- Add 2.0 ml of 5.0 Normality Sodium Hydroxide and mix
- Pour sample into 25 ml graduated cylinder and into clean sample bottle
- Proceed with reactive phosphorus test

- The digestive method is performed prior to tesing for total phosphorus

Phosphorus PhosVer (Ascorbic Acid) Reactive Method

- Gather sample
- Fill a sample cell with one PhosVer 3 Phosphate powder pillow or use AccuVac Ampuls
- Swirl to mix reagent
- Two minute reaction time before testing
- Fill another sample cell with water from sample: This is your blank. Place in the cell holder of the analytical machine
- When timer beeps, the display will show mg/L P PV, press zero and wait for display to show 0.00 mg/L PO4 $^{3-}$
- Place the sample into the machine and press read/enter (DR 4000 will do this automatically)

- If phosphate is present, the sample will turn BLUE in color.

General Quality Control Practices

- Calibration Standards
- Calibration Verification:
  - Check Standard
  - Calibration Blank
- Quality Control Reference Sample
- Batch QC:
  - Method Blank
  - Blank Spike
  - Duplicate Analysis
  - Matrix Spike

Quality Control Limits

- Calibration Curve, r = 0.995 or better
- Check Standard, %Recovery 90-110%
- Method Blank,< reporting limit
- Blank Spike, % Recovery usually 85-115%
- Duplicate Analysis, calculate % RPD
- Matrix Spike, calculate %Recovery & %RPD if analyzed in duplicate

Calculate Bias

- % Recovery

Spike samples, $\%Rec = \frac{(Z - X)}{T} \times 100$

Check Standard or Blank Spike,

$\% Rec = \frac{Y}{T} \times 100$

Where: Z = Concentration in spiked sample
X = Concentration in un-spiked sample
T = True concentration of spike added
Y = Measured concentration

Calculate Precision

- % Relative Percent Difference

$\%RPD = \frac{D}{X} \times 100$

- Where:
- D = Difference between measurements
- X = Mean (Average)

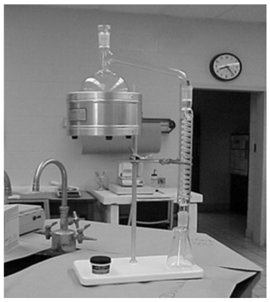
Common Errors

- Sampling error
- Failure to analyze within holding
- Failure to use the correct method
- Failure to follow the method
- Failure to analyze the correct QC
- Calculation errors
- Standards or Reagents prepared incorrectly or expired

Procedural Concerns

- TKN & Total Phosphorus standards should be digested along with samples
- Phosphorus analyses require dedicated glassware

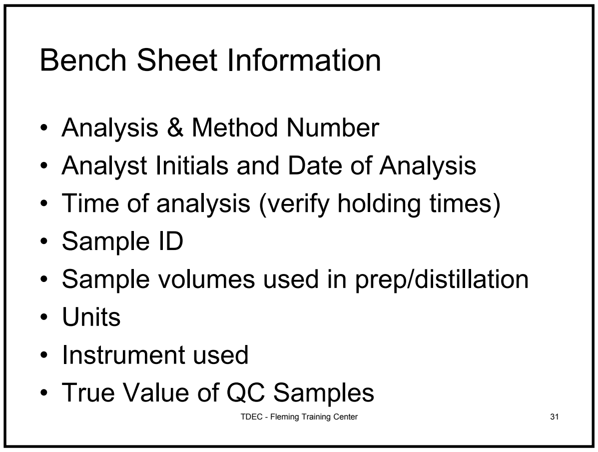
Procedural Concerns



- Ammonia distillation apparatus should be steamed out
- A high & low standard should be carried through the ammonia distillation

Procedural Concerns (cont'd)

- Nitrate-Nitrite, cadmium column should be condition before use
- Verify efficiency of the cadmium column to reduce Nitrate to Nitrite



Bench Sheet Information

- Analysis & Method Number
- Analyst Initials and Date of Analysis
- Time of analysis (verify holding times)
- Sample ID
- Sample volumes used in prep/distillation
- Units
- Instrument used
- True Value of QC Samples

Phosphorus, Reactive (Orthophosphate)

DOC316.53.01119

USEPA[1] PhosVer 3 (Ascorbic Acid) Method[2] **Method 8048**

0.02 to 2.50 mg/L PO_4^{3-} **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater

[1] USEPA Accepted for reporting for wastewater analyses. Procedure is equivalent to USEPA and Standard Method 4500-P-E for wastewater.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Powder pillows			AccuVac Ampuls	
	Sample cell	Cell orientation	Adapter	Sample cell	Adapter
DR 5000	2495402	Fill line faces user	A23618	2427606	A23618
DR 2800	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR 2700	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR/2500	2427606	—	—	2427606	—
DR/2400	2427606	—	—	2427606	—

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

Collect the following items:

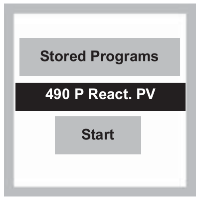
Description	Quantity
Powder Pillow Test:	
PhosVer® 3 Phosphate Reagent powder pillow	1
Sample Cells, 1-inch, 10-mL	2
Stopper for 18 mm Tube (square sample cells only)	1
AccuVac Test:	
Collect at least 40 mL of sample in a 50-mL beaker	40 mL
PhosVer® 3 Phosphate Reagent AccuVac® Ampul	1

Collect the following items: (continued)

Description	Quantity
Beaker, 50-mL	1
Sample Cell, 10-mL round	1
Stopper for 18-mm Tube (supplied with PhosVer AccuVacs)	1

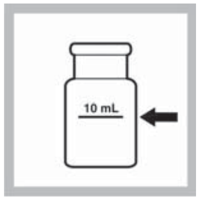
See *Consumables and replacement items* for reorder information.

PhosVer 3 (Ascorbic Acid) method for powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).



2. Fill a sample cell with 10-mL of sample.

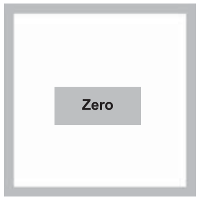
3. **Prepared Sample:** Add the contents of one PhosVer 3 phosphate Powder Pillow to the cell. Immediately stopper and shake vigorously for 30 seconds.

4. Start the instrument timer.

A two-minute reaction period will begin. If the sample was digested using the Acid Persulfate digestion, a ten-minute reaction period is required.

5. **Blank Preparation:** Fill a second sample cell with 10 mL of sample.

6. When the timer expires, wipe the blank and insert it into the cell holder.



7. **ZERO** the instrument.

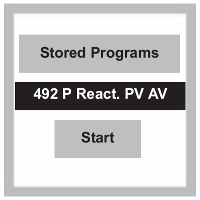
The display will show:

0.00 mg/L PO_4^{3-}

8. Wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L PO_4^{3-}.

PhosVer 3 (Ascorbic Acid) method for AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. **Blank Preparation:** Fill a sample cell with 10-mL of sample.

3. **Prepared Sample:** Fill a PhosVer 3 Phosphate AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.

4. Secure an Ampul cap over the tip of the Ampul. Shake the Ampul for approximately 30 seconds.

Accuracy is unaffected by undissolved powder.

5. Start the instrument timer.

A two-minute reaction period will begin. If the sample was digested using the Acid Persulfate digestion, a ten-minute reaction period is required.

6. When the timer expires, wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show:

0.00 mg/L PO_4^{3-}

7. Wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L PO_4^{3-}.

Interferences

Table 2 Interfering substances

Interfering substance	Interference level
Aluminum	Greater than 200 mg/L
Arsenate	Interferes at any level.
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Hydrogen Sulfide	Interferes at any level
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment. pH 2–10 is recommended.
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Turbidity or color	May cause inconsistent results because the acid in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles. For highly turbid or colored samples, add the contents of one Phosphate Pretreatment[1] Powder Pillow to 25 mL of sample. Mix well. Use this solution to zero the instrument.
Zinc	Greater than 80 mg/L

[1] See *Optional reagents and apparatus.*

Sample collection, preservation and storage

- Collect sample in plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.
- For best results, analyze samples immediately.
- If prompt analysis is not possible, preserve samples by filtering immediately and storing at 4 °C (39 °F) for up to 48 hours.
- Return the sample to room temperature before analysis.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Phosphate 10-mL Ampule Standard, 50-mg/L PO_4^{3-}
- Ampule breaker
- TenSette Pipet

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.

---

* See *Optional reagents and apparatus.*

2. Select standard additions from the instrument menu:

Instrument	Navigate to:
DR 5000	**OPTIONS>MORE>STANDARD ADDITIONS**
DR 2800	**OPTIONS>MORE>STANDARD ADDITIONS**
DR 2700	**OPTIONS>MORE>STANDARD ADDITIONS**
DR/2500	**OPTIONS>STANDARD ADDITIONS**
DR/2400	**OPTIONS>STANDARD ADDITIONS**

3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Prepare a 0.1-mL sample spike by adding 0.1 mL of standard to the unspiked sample. Press the timer icon. After the timer expires, read the result.
6. Prepare a 0.2-mL sample spike by adding 0.1 mL of standard to the 0.1-mL sample spike. Press the timer icon. After the timer expires, read the result.
7. Prepare a 0.3-mL sample spike by adding 0.1 mL of standard to the 0.2-mL sample spike. Press the timer icon. After the timer expires, read the result. Each addition should reflect approximately 100% recovery.

Standard additions method for AccuVac Ampuls (sample spike)
Required for accuracy check:

- Mixing cylinders (3)

1. Fill three mixing cylinders each with 50-mL of sample and spike with 0.2 mL, 0.4 mL and 0.6 mL of standard.
2. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers.
3. Analyze each standard addition sample as described in the *PhosVer 3 (Ascorbic Acid) method for AccuVac® Ampuls*.
4. Accept each standard additions reading. Each addition should reflect approximately 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Phosphate standard solution, 50 mg/L
- Deionized water
- 100-mL Class A volumetric flask
- Class A volumetric pipet
- TenSette Pipet

1. Prepare a 2.00 mg/L phosphate standard solution as follows:
   a. Pipet 4.00 mL of Phosphate Standard, 50-mg/L, into a 100-mL volumetric flask.

   b. Dilute to volume with demineralized water. Mix well. Prepare this solution daily.

Note: *Alternately, use one of the mixed parameter standards listed in Recommended standards. These contain 2.0 mg/L phosphate.*

2. Use this solution in place of the sample. Follow the *PhosVer 3 (Ascorbic Acid) method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	**OPTIONS>MORE>STANDARD ADJUST**
DR 2800	**OPTIONS>MORE>STANDARD ADJUST**
DR 2700	**OPTIONS>MORE>STANDARD ADJUST**
DR/2500	**OPTIONS>STANDARD ADJUST**
DR/2400	**OPTIONS>STANDARD ADJUST**

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
490	DR 5000	2.00 mg/L PO_4^{3-}	1.98–2.02 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}
	DR 2800	2.00 mg/L PO_4^{3-}	1.98–2.02 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}
	DR 2700	2.00 mg/L PO_4^{3-}	1.98–2.02 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}
	DR/2500	1.00 mg/L PO_4^{3-}	0.97–1.03 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}
	DR/2400	1.00 mg/L PO_4^{3-}	0.97–1.03 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
492	DR 5000	2.00 mg/L PO_4^{3-}	1.98–2.02 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}
	DR 2800	2.00 mg/L PO_4^{3-}	1.98–2.02 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}
	DR 2700	2.00 mg/L PO_4^{3-}	1.98–2.02 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}
	DR/2500	1.00 mg/L PO_4^{3-}	0.98–1.02 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}
	DR/2400	1.00 mg/L PO_4^{3-}	0.98–1.02 mg/L PO_4^{3-}	0.02 mg/L PO_4^{3-}

Summary of method

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
PhosVer® 3 Phosphate Reagent Powder Pillows, 10-mL	1	100/pkg	2106069
OR			
PhosVer® 3 Phosphate Reagent AccuVac® Ampuls	1	25/pkg	2508025

Required apparatus (powder pillows)

Description	Quantity/Test	Unit	Catalog number
Stopper for 18 mm Tube	1	6/pkg	173106

Required apparatus (AccuVac)

Description	Quantity/Test	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Stopper for 18 mm Tube	1	6/pkg	173106

Recommended standards

Description	Unit	Catalog number
Phosphate Standard Solution, 10-mL Voluette® Ampul, 50-mg/L as PO_4	16/pkg	17110
Phosphate Standard Solution, 50-mg/L as PO_4	500 mL	17149
Phosphate Standard Solution, 1-mg/L as PO_4	500 mL	256949
Standard, Drinking Water, Mixed Parameter, Inorganic: F, NO_3, PO_4, SO_4	500 mL	2833049
Wastewater Effluent Standard, for mixed parameters: NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Hydrochloric Acid Solution, 6.0N, 1:1	500 mL	88449
Mixing Cylinder 50 mL	each	189641
Phosphate Treatment Powder Pillow	100/pkg	1450199
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
Pipet, TenSette, Pipet, 1.0 - 10.0 mL	each	1970010
Pipet Tips, for TenSette Pipet 1970010[1]	50/pkg	2199796
Pipet Tips, for TenSette Pipet 1970010[1]	250/pkg	2199725
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076

Optional reagents and apparatus

Description	Unit	Catalog number
pH Paper, 0–14 pH range	100/pkg	2601300
AccuVac snapper	each	2405200
AccuVac ampule blanks	25/pkg	2677925
Flask, volumetric, 100 mL	each	1457442
Pipet, volumetric, Class A, 4 mL	each	1451504
AccuVac ampule drainer	each	4103600

[1] Other sizes are available

Optional standards

Description	Unit	Catalog number
Voluette Ampule breaker 10 mL	each	2196800
Phosphate, 10 mg/L	946 mL	1420416
Phosphate, 15 mg/L	100 mL	1424342
Phosphate; 100 mg/L	100 mL	1436832
Phosphate; 500 mg/L, 10 mL Voluette Ampules	16/pkg	1424210
Phosphate; 500 mg/L	100 mL	1424232

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Updated February 2008, Edition 5

Phosphorus, Total

DOC316.53.01121

USEPA[1]
PhosVer® 3 with Acid Persulfate Digestion Method

Method 8190

0.06 to 3.50 mg/L PO_4^{3-} or
0.02 to 1.10 mg/L P

Test 'N Tube™ Vials

Scope and Application: For water, wastewater and seawater

[1] USEPA Accepted for reporting wastewater analyses (Standard Methods 4500 P-E).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Light shield	Adapter
DR 5000	—	—
DR 2800	LZV646	—
DR 2700	LZV646	—
DR/2500	—	—
DR/2400	—	5945700

Before starting the test:

DR 2800 and DR 2700 only: Install the light shield in Cell Compartment #2 before performing this test.

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

The test range for total phosphate is limited to 0.06 to 3.5 mg/L PO_4^{3-}. Values greater than 3.5 mg/L may be used to estimate dilution ratios, but should NOT be used for reporting purposes. If the value is greater than 3.5 mg/L, dilute the sample and repeat the digestion and the colorimetric test.

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Refer to the current MSDS for safe handling and disposal instructions.

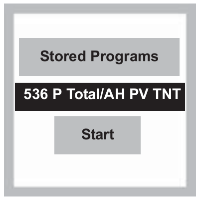
Collect the following items:

Description	Quantity
Total Phosphorus Test 'N Tube™ Reagent Set	1
Deionized water	varies
DRB200 Reactor	1
Funnel, micro	1
Light Shield or Adapter (see *Instrument-specific information*)	1
Pipet, TenSette®, 1 to 10 mL, plus tips	1
Test Tube Rack	1

See *Consumables and replacement items* for reorder information.

PhosVer 3, acid persulfate digestion

1. Turn on the DRB200 Reactor. Preheat to 150 °C.

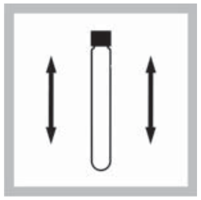


2. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

3. Use a TenSette® Pipet to add 5.0 mL of sample to a Total Phosphorus Test Vial.

4. Use a funnel to add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to the vial.

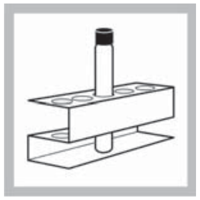


5. Cap tightly and shake to dissolve.

6. Insert the vial into the DRB200. Close the protective cover.

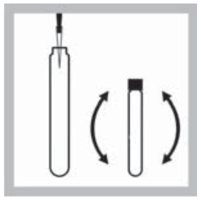
7. Set the instrument timer to 30 minutes and start.

A 30-minute heating period will begin.



8. When the timer expires, carefully remove the hot vial from the reactor. Insert it in a test tube rack and cool to room temperature.

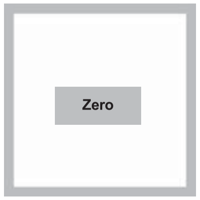
PhosVer 3, acid persulfate digestion (continued)



9. Use a TenSette Pipet to add 2 mL of 1.54 N Sodium Hydroxide Standard Solution to the vial. Cap and mix.

10. Wipe the outside of the vial with a damp cloth followed by a dry one.

11. Insert the vial into the 16 mm cell holder.

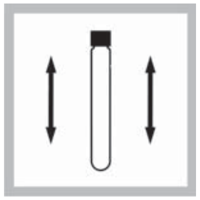


12. **ZERO** the instrument.

The display will show:

0.00 mg/L PO_4^{3-}

13. Use a funnel to add the contents of one PhosVer 3 Powder Pillow to the vial.



14. Immediately cap tightly and shake to mix for 20–30 seconds.

The powder will not dissolve completely.

15. Start the instrument timer.

A two-minute reaction period will begin.

Read the sample within 2–8 minutes after the timer expires.

16. After the timer expires, wipe the outside of the vial with a wet towel, then a dry one. Insert the prepared sample vial into the 16 mm cell holder.

READ the results in mg/L PO_4^{3-}.

Interferences

Table 2 Interfering substances

Interfering substance	Interference level
Aluminum	Greater than 200 mg/L
Arsenate	Interferes at any level
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Silica	Greater than 50 mg/L

Table 2 Interfering substances (continued)

Interfering substance	Interference level
Silicate	Greater than 10 mg/L
Sulfide	Greater than 90 mg/L
Turbidity or color	May cause inconsistent results because the acid in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Zinc	Greater than 80 mg/L

Sample collection, preservation and storage

- Collect samples in plastic or glass bottles that have been acid washed with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use commercial detergents containing phosphate for cleaning glassware used in this test.
- Analyze the samples immediately for the most reliable results.
- If prompt analysis is not possible, samples may be preserved up to 28 days by adjusting the pH to 2 or less with concentrated Sulfuric Acid* (about 2 mL per liter) and storing at 4 °C.
- Warm stored samples to room temperature and neutralize with 5.0 N Sodium Hydroxide* before analysis.
- Correct test results for volume additions.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Phosphate 10-mL Ampule Standard, 50-mg/L as PO_4^{3-}
- Ampule breaker
- TenSette Pipet
- Mixing cylinders, (3)

1. Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.
2. After reading test results, leave the sample cell (unspiked sample) in the instrument.
3. Select standard additions from the instrument menu:

Instrument	Navigate to:
DR 5000	**OPTIONS>MORE>STANDARD ADDITIONS**
DR 2800	**OPTIONS>MORE>STANDARD ADDITIONS**
DR 2700	**OPTIONS>MORE>STANDARD ADDITIONS**
DR/2500	**OPTIONS>STANDARD ADDITIONS**
DR/2400	**OPTIONS>STANDARD ADDITIONS**

4. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.

---

* See *Optional reagents and apparatus.*

5. Open the standard solution ampule.
6. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample.
7. Use a 5-mL aliquot of the spiked sample in place of the sample. Follow the *PhosVer 3, acid persulfate digestion* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
8. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- Phosphate standard solution, 3.0-mg/L

1. Use the 3.0 mg/L phosphate standard solution in place of the sample. Follow the *PhosVer 3, acid persulfate digestion* test procedure.
2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	**OPTIONS>MORE>STANDARD ADJUST**
DR 2800	**OPTIONS>MORE>STANDARD ADJUST**
DR 2700	**OPTIONS>MORE>STANDARD ADJUST**
DR/2500	**OPTIONS>STANDARD ADJUST**
DR/2400	**OPTIONS>STANDARD ADJUST**

3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
536	DR 5000	3.00 mg/L PO_4^{3-}	2.93–3.07 mg/L PO_4^{3-}	0.06 mg/L PO_4^{3-}
	DR 2800	3.00 mg/L PO_4^{3-}	2.93–3.07 mg/L PO_4^{3-}	0.06 mg/L PO_4^{3-}
	DR 2700	3.00 mg/L PO_4^{3-}	2.93–3.07 mg/L PO_4^{3-}	0.06 mg/L PO_4^{3-}
	DR/2500	3.00 mg/L PO_4^{3-}	2.90–3.10 mg/L PO_4^{3-}	0.06 mg/L PO_4^{3-}
	DR/2400	3.00 mg/L PO_4^{3-}	2.90–3.10 mg/L PO_4^{3-}	0.06 mg/L PO_4^{3-}

Summary of method

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphates by heating with acid and persulfate.

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Total Phosphorus Test 'N Tube™ Reagent Set, 50 tests, includes:	—	—	2742645
PhosVer® 3 Phosphate Reagent Powder Pillows	1	50/pkg	2106046
Potassium Persulfate Powder Pillows	1	50/pkg	2084766
Sodium Hydroxide Solution, 1.54 N	2 mL	100 mL	2743042
Total and Acid Hydrolyzable Test Vials[1]	1	50/pkg	—
Water, deionized	varies	100 mL	27242

[1] Not sold separately

Required apparatus

Description	Quantity/Test	Unit	Catalog number
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Funnel, micro	1	each	2584335
Pipet, TenSette®, 1.0 to 10 mL	1	each	1970010
Pipet Tips for TenSette Pipet 19700-10	1	250/pkg	2199725
Test Tube Rack	1	each	1864100

Recommended standards

Description	Unit	Catalog number
Drinking Water Standard, Mixed Parameter, Inorganic for F-, NO_3, PO_4, SO_4	500 mL	2833049
Phosphate Standard Solution, 10-mL Voluette® Ampule, 50-mg/L as PO_4^{3-}	16/pkg	17110
Phosphate Standard Solution, 1-mg/L as PO_4^{3-}	500 mL	256949
Phosphate Standard Solution, 3 mg/L as PO_4^{3-}	946 mL	2059716
Wastewater Standard, Effluent Inorganics, for NH_3–N, NO_3–N, PO_4, COD, SO_4, TOC	500 mL	2833249
Voluette Ampule breaker 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing	25 mL	189640
Pipet, volumetric, Class A, 2.00 mL	1	each
Hydrochloric Acid Solution, 6.0 N, 1:1	500 mL	88449
Sodium Hydroxide, 5.0 N	1000 mL	245053
Sulfuric Acid, concentrated	500 mL	97949
Pipet, TenSette® Pipet, 0.1–1.0 mL	each	1970001

Optional reagents and apparatus (continued)

Description	Unit	Catalog number
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
pH Paper, 0–14 pH range	100/pkg	2601300
Deionized Water	4 L	27256
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Finger cots	2/pkg	1464702

Optional standards

Description	Unit	Catalog number
Phosphate, 10 mg/L	946 mL	1420416
Phosphate, 15 mg/L	100 mL	1424342
Phosphate, 100 mg/L	100 mL	1436832
Phosphate, 500 mg/L, 10 mL Voluette Ampules	16/pkg	1424210
Phosphate, 500 mg/L	100 mL	1424232

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Updated February 2008, Edition 5

Phosphorus, Total, Digestion

DOC316.53.01112

USEPA[1] Acid Persulfate Digestion Method[2]

Method 8190

Scope and Application: For water, wastewater and seawater

[1] USEPA Accepted for wastewater analyses when used with the ascorbic acid (PhosVer 3) method.

[2] Adapted from *Standard Methods for the Examination of Water and Wastewater* 4500-P B & E

Test preparation

Before starting the test:

Rinse all glassware with 1:1 hydrochloric acid. Rinse again with deionized water.

Collect the following items:

Description	Quantity
Potassium Persulfate Powder Pillows	1
Sodium Hydroxide Solution, 5.0 N	2 mL
Sulfuric Acid Solution, 5.25 N	2 mL
Water, deionized	varies
Cylinder, graduated, 25-mL	1
Flask, Erlenmeyer, 125-mL	1
Hot Plate	1

See *Consumables and replacement items* for reorder information.

Acid Persulfate Digestion

1. Use a graduated cylinder to measure 25 mL of sample. Pour the sample into a 125-mL Erlenmeyer flask.

2. Add the contents of one Potassium Persulfate Powder Pillow.

Swirl to mix.

3. Use a 1-mL calibrated dropper to add 2.0 mL of 5.25 N Sulfuric Acid Solution to the flask.

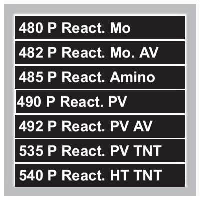
4. Place the flask on a hot plate. Boil gently for 30 minutes. Do not boil dry.

Concentrate the sample to less than 20 mL for best recovery. After concentration, maintain the volume near 20 mL by adding small amounts of deionized water. Do not exceed 20 mL.

5. Cool the sample to room temperature.

6. Use a 1-mL calibrated dropper to add 2.0 mL of 5.0 N Sodium Hydroxide Solution to the flask. Swirl to mix.

7. Pour the sample into a 25-mL graduated cylinder. Adjust the volume to 25 mL with deionized water rinsings from the flask.



480 P React. Mo
482 P React. Mo. AV
485 P React. Amino
490 P React. PV
492 P React. PV AV
535 P React. PV TNT
540 P React. HT TNT

8. Proceed with a reactive phosphorus test of the expected total phosphorus concentration range.

Extend the color development time to 10 minutes for the PhosVer 3 (ascorbic acid) method.

Interferences

Table 1 Interfering substances

Interfering substance	Interference level
Alkaline or highly buffered samples	It may be necessary to add additional acid in step 3 to drop the pH of the solution below 1.
Turbidity	Use 50 mL of sample and double the reagent quantities. Use a portion of the reacted sample to zero the instrument in the reactive phosphorus procedure. This compensates for any color or turbidity destroyed by this procedure.

Sample collection, preservation and storage

- Analyze the samples immediately for the most reliable results.
- If prompt analysis is not possible, samples may be preserved up to 28 days.
- To preserve samples. adjust the pH to 2 or less with Concentrated Sulfuric Acid* (about 2 mL per liter). Store at 4 °C.
- Warm the sample to room temperature and neutralize with 5.0 N Sodium Hydroxide before analysis.
- Correct test results for volume additions.

Summary of method

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphate by heating with acid and persulfate. Organically bound phosphates are thus determined indirectly by subtracting the result of an acid hydrolyzable phosphorus test from the total phosphorus result.

This procedure must be followed by one of the reactive phosphorus (orthophosphate) analysis methods for determining the phosphorus content of the sample. If the ascorbic acid (PhosVer 3) method is used to measure the reactive phosphorus, this method is USEPA accepted for NPDES reporting.

The following reagents and apparatus are required in addition to those required for the active phosphorus test.

---

* See *Optional reagents*.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Potassium Persulfate Powder Pillows	1	100/pkg	245199
Sodium Hydroxide Solution, 5.0 N	2 mL	100 mL MDB	245032
Sulfuric Acid Solution, 5.25 N	2 mL	100 mL MDB	244932
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Cylinder, graduated, 25-mL	1	each	50840
Flask, Erlenmeyer, 125-mL	1	each	50543
Hot Plate, 7” x 7” digital, 240 VAC	1	each	2881500
Hot Plate, 7” x 7” digital, 240 VAC	1	each	2881502

Optional reagents

Description	Unit	Catalog number
Sodium, Hydroxide, 5.0 N	1000 mL	245053
Sulfuric Acid, concentrated	500 mL	97949
pH paper, 0–14 pH range	100/pkg	2601300
Thermometer, Non-Mercury, –10 to 225°C	each	2635700
Sampling bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
Hydrochloric Acid, 6.0 N	500 mL	88449

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Updated February 2008, Edition 5

Nitrogen, Total

DOC316.53.001086

Persulfate Digestion Method **Method 10071**

LR (0.5 to 25.0 mg/L N) **Test 'N Tube™ Vials**

Scope and Application: For water and wastewater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Light shield	Adapter
DR 5000	—	—
DR 2800	LZV646	—
DR 2700	LZV646	—
DR/2500	—	—
DR/2400	—	5945700

Before starting the test:

DR 2800 and DR 2700 only: Install the light shield in Cell Compartment #2 before performing this test.
Digestion is required for determining total nitrogen.
This test is technique-sensitive. Invert the vials as described here to avoid low results: Hold the vial in a vertical position with the cap pointing up. Turn the vial upside-down. Wait for all of the solution to flow down to the cap. Pause. Return the vial to an upright position. Wait for all the solution to flow to the bottom of the vial. This process equals one inversion.
If the test overranges, repeat the digestion and measurement with diluted sample. The digestion must be repeated for accurate results.
Use the deionized water provided in the reagent set or Organic-free Water to prepare the standards and perform the procedure.

Collect the following items:

Description	Quantity
Test 'N Tube™ LR Total Nitrogen Reagent Set	1
DRB200 Reactor	1
Funnel, micro	1
Light Shield or adapter (see *Instrument-specific information*)	1
Pipet, TenSette®, 1.0 to 10.0 mL plus tips	1
Test Tube Cooling Rack	1–3
Finger Cots	2

See *Consumables and replacement items* for reorder information.

Persulfate digestion method

1. Turn on the DRB200 Reactor and heat to 105 °C.

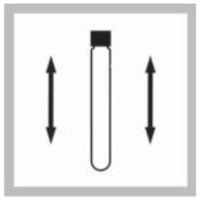
2. Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two Total Nitrogen Hydroxide Digestion Reagent vials. Wipe off any reagent that may get on the lid or the tube threads.

***Note:** One reagent blank is sufficient for each set of samples.*

3. **Prepared Sample:** Add 2 mL of sample to one vial.

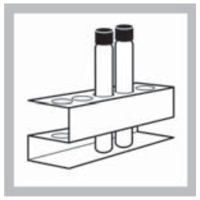
Blank Preparation: Add 2 mL of the deionized water included in the kit to a second vial.

Use only water that is free of all nitrogen-containing species as a substitute for the provided deionized water.

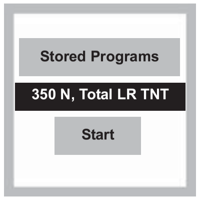


4. Cap both vials. Shake vigorously for at least 30 seconds to mix. The persulfate reagent may not dissolve completely after shaking. This will not affect accuracy.

5. Insert the vials in the reactor and close the lid. **Heat for exactly 30 minutes.**



6. Using finger cots, **immediately** remove the hot vials from the reactor. Cool the vials to room temperature.

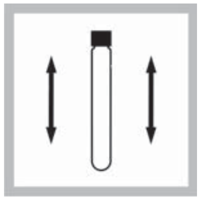


7. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

8. Remove the caps from the digested vials and add the contents of one Total Nitrogen (TN) Reagent A Powder Pillow to each vial.

Persulfate digestion method (continued)

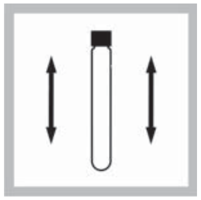


9. Cap the tubes and shake for 15 seconds.

10. Start the instrument timer.

A three-minute reaction period will begin.

11. After the timer expires, remove the caps from the vials and add one TN Reagent B Powder Pillow to each vial.

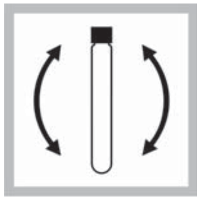


12. Cap the tubes and shake for 15 seconds. The reagent will not completely dissolve. This will not affect accuracy. The solution will begin to turn yellow.

13. Start the instrument timer.

A two-minute reaction period will begin.

14. After the timer expires, remove the caps from two TN Reagent C vials and add 2 mL of digested, treated sample to one vial. Add 2 mL of digested, treated reagent blank to the second TN Reagent C vial.



15. Cap the vials and invert ten times to mix. Use slow, deliberate inversions for complete recovery.

The tubes will be warm to the touch.

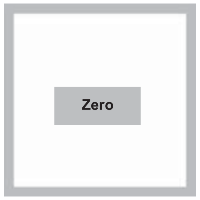
16. Start the instrument timer.

A five-minute reaction period will begin.

The yellow color will intensify.

Persulfate digestion method (continued)

17. Wipe the reagent blank and insert it into the 16-mm round cell holder.



18. **ZERO** the instrument.

The display will show:

0.0 mg/L N

19. Wipe the reagent vial and insert it into the 16-mm round cell holder.

Note: *Multiple samples may be read after zeroing on one reagent blank.*

20. **READ** the results in mg/L N.

Blanks for colorimetric measurement

The reagent blank may be used up to seven days for measurements using the same lots of reagents. Store it in the dark at room temperature (18–25 °C). If a small amount of white floc appears within a week, discard the reagent blank and prepare a new one.

Interferences

The *Non-interfering substances* table shows substances that have been tested and found not to interfere up to the indicated levels (in mg/L). Interfering substances that resulted in a concentration change of ±10% appear in the *Interfering substances* table.

Table 2 Non-interfering substances

Interfering substance	Interference level
Barium	2.6 mg/L
Calcium	300 mg/L
Chromium ($^{3+}$)	0.5 mg/L
Iron	2 mg/L
Lead	6.6 µg/L
Magnesium	500 mg/L
Organic Carbon	150 mg/L
pH	13 pH units
Phosphorus	100 mg/L
Silica	150 mg/L
Silver	0.9 mg/L
Tin	1.5 mg/L

Table 3 Interfering substances

Interfering substance	Interference level
Bromide	> 60 mg/L; positive interference

Table 3 Interfering substances (continued)

Interfering substance	Interference level
Chloride	> 1000 mg/L; positive interference

This test performed with standard nitrogen solutions prepared from the following compounds obtained 95% recovery:

- Ammonium chloride
- Ammonium sulfate
- Ammonium acetate
- Urea
- Glycine

Ammonium chloride or nicotinic-PTSA spikes in domestic influent, effluent and the ASTM standard specification for substitute wastewater (D 5905-96) also resulted in ≥ 95% recovery.

The large amounts of nitrogen-free organic compounds in some samples may decrease digestion efficiency by consuming some of the persulfate reagent. Samples known to contain high levels of organics should be diluted and re-run to verify digestion efficiency.

Sample collection, storage and preservation

- Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.
- Preserve the sample by reducing the pH to 2 or less with concentrated (at least 2 mL/L) Sulfuric Acid.
- Store samples at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days.
- Warm stored samples to room temperature and neutralize with 5 N Sodium Hydroxide before analysis.
- Correct the test result for volume additions.

Accuracy check

This method generally yields 95–100% recovery on organic nitrogen standards. For proof of accuracy use Primary Standards for Kjeldahl Nitrogen.

1. Prepare one or more of the following three solutions. Each preparation is for an equivalent 25-mg/L N standard. Use the deionized water included in the kit or water that is free of all organic and nitrogen-containing species.
   - **a.** Weigh 0.3379 g of Ammonium p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
   - **b.** Weigh 0.4416 g of Glycine p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
   - **c.** Weigh 0.5274 g of Nicotinic p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
2. Analyze each of these solutions using the test procedure above. Calculate the percent recovery for each using this formula. Refer to the *Percent recovery* table for more information.

$$\%\ \text{recovery} = \frac{\text{measured concentration}}{25} \times 100$$

Refer to the *Percent recovery* table.

Table 4 Percent recovery

Compound	Lowest Expected % Recovery
Ammonia-PTSA	95%
Glycine-PTSA	95%
Nicotinic-PTSA	95%

Analysts have found Ammonia-PTSA to be the most difficult to digest. Other compounds may yield different percent recoveries.

Standard additions method (sample spike)
Required for accuracy check:

- Ammonia Nitrogen Standard Solution, 1000-mg/L as NH_3–N
- Ampule breaker
- TenSette Pipet
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu:

Instrument	Navigate to:
DR 5000	**OPTIONS>MORE>STANDARD ADDITIONS**
DR 2800	**OPTIONS>MORE>STANDARD ADDITIONS**
DR 2700	**OPTIONS>MORE>STANDARD ADDITIONS**
DR/2500	**OPTIONS>STANDARD ADDITIONS**
DR/2400	**OPTIONS>STANDARD ADDITIONS**

3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample.
6. Follow the *Persulfate digestion method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 10-mg/L ammonia nitrogen standard solution

1. Substitute 2 mL of a 10-mg/L ammonia nitrogen standard solution in place of the sample. Follow the *Persulfate digestion method* test procedure.

2. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	**OPTIONS>MORE>STANDARD ADJUST**
DR 2800	**OPTIONS>MORE>STANDARD ADJUST**
DR 2700	**OPTIONS>MORE>STANDARD ADJUST**
DR/2500	**OPTIONS>STANDARD ADJUST**
DR/2400	**OPTIONS>STANDARD ADJUST**

3. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—∆Concentration per 0.010 ∆Abs
350	DR 5000	10 mg/L NH_3–N	9.6–10.4 mg/L N	0.5 mg/L N
350	DR 2800	10 mg/L NH_3–N	9.6–10.4 mg/L N	0.5 mg/L N
350	DR 2700	10 mg/L NH_3–N	9.6–10.4 mg/L N	0.5 mg/L N
350	DR/2500	10 mg/L NH_3–N	9.0–11.0 mg/L N	0.5 mg/L N
350	DR/2400	10 mg/L NH_3–N	9.0–11.0 mg/L N	0.5 mg/L N

Summary of method

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
Test 'N Tube™ Total Nitrogen Reagent Set, LR	50 vials	2672245

Required apparatus (powder pillows)

Description	Quantity/Test	Unit	Catalog number
DRB200 Reactor, 110 V, 15x16 mm	1	each	LTV082.53.40001
DRB200 Reactor, 220 V, 15x16 mm	1	each	LTV082.52.40001
Funnel, micro	1	each	2584335
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 19700-10	2	50/pkg	2199796
Test Tube Cooling Rack	1–3	each	1864100
Finger Cots	2	2/pkg	1464702

Recommended standards

Description	Unit	Catalog number
Ammonia Nitrogen Standard Solution, 1000-mg/L NH_3–N	1 L	2354153
Ammonia Nitrogen Standard Solution, 10-mg/L NH_3–N	500 mL	15349
Primary Standard Set, for Kjeldahl Nitrogen	set of 3	2277800
Wastewater Mixed Inorganic Standard for NH_3-H, NO_3-N, PO4, COD, SO_4,TOC	500 mL	2833149
Water, deionized	500 mL	27249
Water, organic-free	500 mL	2641549

Optional reagents and apparatus

Description	Unit	Catalog number
Balance, analytical, 80 g capacity, 115 VAC	each	2936701
Cylinder, mixing with stopper, 50 mL	each	2088641
Flask, volumetric, Class A, 1000 mL	each	1457453
Pipet, TenSette, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet tips for TenSette Pipet 1970001	1000/pkg	2185628
Pipet tips for TenSette Pipet 1970010	250-pkg	2199725
Sodium Hydroxide, 5 N	50 mL	245026
Sulfuric Acid, concentrated	500 mL	97949
PourRite® Ampule breaker, 2-mL	each	2484600
Voluette® Ampule breaker 10 mL	each	2196800
Ammonia Nitrogen Standard Solution, 1-mg/L NH_3–N	500 mL	189149
Ammonia Nitrogen Standard Solution, 100-mg/L NH_3–N	500 mL	2406549
Ammonia Nitrogen Standard Solution, 2-mL PourRite Ampule, 50 mg/L	20/pkg	1479120
Ammonia Nitrogen Standard Solution, 10-mL Voluette Ampules, 10 mg/L	16/pkg	1479110
Ammonia Nitrogen Standard Solution, 10-mL Voluette Ampules, 150 mg/L	16/pkg	2128410
Ammonia Nitrogen Standard Solution, 10-mL Voluette Ampules, 160 mg/L	16/pkg	2109110

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Updated February 2008, Edition 5

Nitrate

DOC316.53.01069

Cadmium Reduction Method **Method 8171**

MR (0.1 to 10.0 mg/L NO_3^-–N) **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Powder pillows			AccuVac Ampuls	
	Sample cell	Cell orientation	Adapter	Sample cell	Adapter
DR 5000	2495402	Fill line faces user	A23618	2427606	A23618
DR 2800	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR 2700	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR/2500	2427606	—	—	2427606	—
DR/2400	2427606	—	—	2427606	—

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample.

A deposit of unoxidized metal will remain at the bottom of the cell after the NitraVer® 5 dissolves. The deposit will not affect results.

This method is technique-sensitive. Shaking time and technique influence color development. For most accurate results, make successive tests on a 10.0-mg/L Nitrate Nitrogen Standard solution. Adjust shaking times to obtain the correct result.

Rinse the sample cell immediately after use to remove all cadmium particles. Retain the used sample for proper hazardous waste disposal for cadmium.

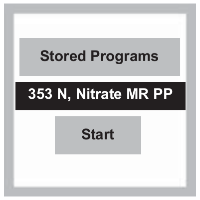
Prepared samples will contain cadmium and must be disposed of according to Federal, State and local hazardous waste regulations. Refer to the current MSDS for safe handling and disposal instructions.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
NitraVer® 5 Nitrate Reagent Powder Pillow	1
Sample Cells (see *Instrument-specific information*)	2
Stopper, Neoprene #2, solid	2
AccuVac Test:	
Collect at least 40 mL of sample in a 50-mL beaker	40 mL
NitraVer® 5 Nitrate Reagent AccuVac® Ampul	1
Beaker, 50-mL (AccuVac test)	1
Sample Cell for blank (see *Instrument-specific information*)	1

See *Consumables and replacement items* for reorder information.

Cadmium reduction method for powder pillows



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Fill a sample cell with 10 mL of sample.

3. **Prepared Sample:** Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow. Insert a stopper into the cell.

4. Start the instrument timer.

A one-minute reaction period will begin.

Shake the cell vigorously until the timer expires.
Note: *Some solid material will not dissolve.*

Cadmium reduction method for powder pillows (continued)

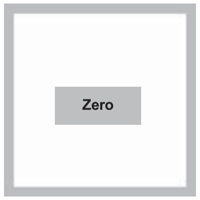
5. When the timer expires, start the timer again.

A five-minute reaction period will begin.

An amber color will develop if nitrate is present.

6. Blank Preparation: When the timer expires, fill a second sample cell with 10 mL of sample.

7. Wipe and insert the blank into the cell holder.



8. ZERO the instrument.

The display will show:

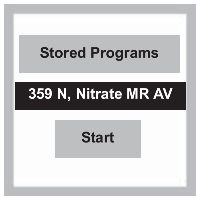
0.0 mg/L NO_3^-–N

9. Within two minutes after the timer expires, wipe and insert the prepared sample into the cell holder.

10. READ the results in mg/L 0.0 mg/L NO_3^-–N.

Refer to the user manual to display other chemical forms.

Cadmium reduction method for AccuVac® Ampuls



1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Prepared Sample: Collect at least 40 mL of sample in a 50-mL beaker.

3. Fill a NitraVer 5 Nitrate AccuVac® Ampul with sample. Keep the tip immersed while the Ampul fills completely. Place a stopper over the Ampul tip.

4. Start the instrument timer.

A one-minute reaction period will begin.

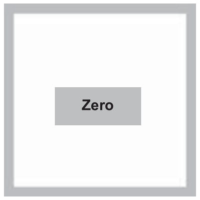
5. Invert the Ampul 48–52 times as the timer counts down.

6. When the timer expires, start the timer again.

A five-minute reaction period will begin. An amber color will develop if nitrate is present.

7. Blank Preparation: When the timer expires, fill a round sample cell with 10 mL of sample.

8. Wipe the blank and insert it into the cell holder.

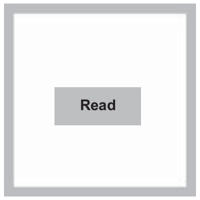


9. ZERO the instrument.

The display will show:

0.0 mg/L NO_3^-–N

10. Within two minutes after the timer expires, wipe the Ampul and insert it into the cell holder.



11. READ the results in mg/L NO_3^-–N.

Interferences

Table 2 Interfering substances

Interfering substance	Interference level
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration (see *Seawater calibration*).
Ferric iron	Interferes at all levels
Nitrite	Interferes at all levels Compensate for nitrite interference as follows: **1.** Add 30-g/L Bromine Water[1] drop-wise to the sample until a yellow color remains. **2.** Add one drop of 30-g/L Phenol Solution[1] to destroy the color. **3.** Proceed with Step 2 of the test. Report the results as total nitrate and nitrite.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

[1] See *Optional reagents and apparatus.*

Seawater calibration

Chloride concentrations above 100 mg/L will cause low results. To perform this test in water with high interference level, calibrate the water using standards spiked to the same chloride concentrations as the required samples. To prepare calibration standards containing 0.06, 0.1, 0.3 and 0.4 mg/L nitrate as NO_3–N:

1. Prepare a 1 L volume of chloride water that matches the concentration of the samples, using the following equation:
   - **a.** Add necessary Chloride concentration (g/L) x (1.6485) = g of ACS grade NaCl to 1 L of deionized water. (

   Note: *18.8 g/L is a typical seawater chloride concentration.*

   - **b.** Mix this solution thoroughly to make sure that it is a homogeneous solution. Use this water as the dilution water instead of the deionized water when preparing the nitrate standards.
2. Use Class A glassware or a Tensette Pipet to pipet 0.6, 1, 3 and 4 mL of the 10 mg/L Nitrogen-Nitrate as NO_3^-–N (NIST) Standard Solution (Catalog Number 30749) into four different 100 mL Class A volumetric flasks.
3. Dilute to the mark with the prepared chloride water. Mix thoroughly.
4. Use the prepared chloride water for the 0-mg/L nitrate as NO_3–N standard.

Sample collection, preservation and storage

- Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 24 hours at 4 °C. To preserve samples for longer periods, add 2 mL of Concentrated Sulfuric Acid (H_2SO_4)* per liter and store at 4 °C. The results are reported as total nitrate and nitrite.
- Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution*. Do not use mercury compounds as preservatives.

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* See *Optional reagents and apparatus.*

- Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Nitrate Nitrogen Standard,100-mg/L NO_3^-–N
- TenSette Pipet and Pipet Tips

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu:

Instrument	Navigate to:
DR 5000	**OPTIONS>MORE>STANDARD ADDITIONS**
DR 2800	**OPTIONS>MORE>STANDARD ADDITIONS**
DR 2700	**OPTIONS>MORE>STANDARD ADDITIONS**
DR/2500	**OPTIONS>STANDARD ADDITIONS**
DR/2400	**OPTIONS>STANDARD ADDITIONS**

3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution bottle.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample.
6. Follow the *Cadmium reduction method for powder pillows* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard additions method for AccuVac Ampuls (sample spike)
Required for accuracy check:

- 500 mg/L Nitrate Nitrogen Ampule Standard Solution
- Ampule breaker
- TenSette Pipet and Pipet Tips
- Mixing cylinder, 50-mL (3)

1. Fill three mixing cylinders each with 50-mL of sample and spike with 0.1 mL, 0.2 mL and 0.3 mL of 500 mg/L Nitrate Nitrogen Ampule Standard Solution.
2. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers.
3. Analyze each standard addition sample as described in the *Cadmium reduction method for AccuVac® Ampuls*.
4. Accept each standard additions reading. Each addition should reflect approximately 100% recovery. Standard solution method

Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 5.0-mg/L Nitrate Nitrogen Standard Solution (prepared)
- 100-mg/L Nitrate Nitrogen Standard
- Deionized water
- 100-mL volumetric flask
- 5-mL Volumetric pipet
- TenSette Pipet and Pipet Tips

1. Prepare a 5.0-mg/L nitrate nitrogen standard solution as follows:
   a. Pipet 5.0 mL of 100-mg/L Nitrate Nitrogen Standard, into a 100-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well.
2. Use this 5.0 mg/L nitrate nitrogen standard solution in place of the sample. Follow the *Cadmium reduction method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	**OPTIONS>MORE>STANDARD ADJUST**
DR 2800	**OPTIONS>MORE>STANDARD ADJUST**
DR 2700	**OPTIONS>MORE>STANDARD ADJUST**
DR/2500	**OPTIONS>STANDARD ADJUST**
DR/2400	**OPTIONS>STANDARD ADJUST**

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
353	DR 5000	5.0 mg/L NO_3^-–N	4.8–5.2 mg/L NO_3^-–N	0.04 mg/L NO_3^-–N
	DR 2800			
	DR 2700			
	DR/2500	10 mg/L NO_3^-–N	8.0–12.0 mg/L NO_3^-–N	0.1 mg/L NO_3^-–N
	DR/2400			
359	DR 5000	5.0 mg/L NO_3^-–N	4.6–5.4 mg/L NO_3^-–N	0.05 mg/L NO_3^-–N
	DR 2800			
	DR 2700			
	DR/2500	10 mg/L NO_3^-–N	9.2–10.8 mg/L NO_3^-–N	0.1 mg/L NO_3^-–N
	DR/2400			

Summary of method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber colored solution. Test results are measured at 400 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
NitraVer® 5 Nitrate Reagent Powder Pillows (for 10 mL sample)	1	100/pkg	2106169
OR			
NitraVer® 5 Nitrate Reagent AccuVac® Ampul	1	25/pkg	2511025

Required apparatus (powder pillows)

Description	Quantity/Test	Unit	Catalog number
Stopper, Neoprene, solid, size no. 2	2	12/pkg	1480802

Required apparatus (AccuVac)

Description	Quantity/Test	Unit	Catalog number
Beaker, 50-mL	1	each	50041H

Recommended standards

Description	Unit	Catalog number
Mixed Parameter Drinking Water Standard, for F, NO_3–N, PO_4, SO_4	500 mL	2833049
Nitrate Nitrogen Standard Solution, 100-mg/L NO_3^-–N	500 mL	194749
Nitrate Nitrogen Standard Solution, 500 mg/L NO_3–N, 10-mL ampules	16/pkg	1426010
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Bromine Water, 30-mg/L	29 mL	221120
Cylinder, mixing, 50 mL	each	2088641
Flask, volumetric, 100-mL	each	1457442
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet, volumetric, 5.00 mL	each	1451537
Pipet Filler, safety bulb	each	1465100
Phenol Solution, 30-g/L	29 mL	211220
5.0 N Sodium Hydroxide Standard Solution	1 L	245053
Sulfuric Acid, concentrated	500 mL	97949

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Updated February 2008, Edition 5

Nitrite

DOC316.53.01074

USEPA[1] Diazotization **Method 8507**

LR (0.002 to 0.300 mg/L NO_2^-–N) **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater

[1] USEPA approved for wastewater analysis, Federal Register, 44(85), 25505 (May 1, 1979)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Powder pillows			AccuVac Ampuls	
	Sample cell	Cell orientation	Adapter	Sample cell	Adapter
DR 5000	2495402	Fill line faces user	A23618	2427606	A23618
DR 2800	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR 2700	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR/2500	2427606	—	—	2427606	—
DR/2400	2427606	—	—	2427606	—

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
NitriVer® 3 Nitrite Reagent Powder Pillows	1
Sample Cells (see *Instrument-specific information*)	2
AccuVac Test:	
NitriVer® 3 Nitrite Reagent AccuVac® Ampul.	1
Beaker, 50-mL	1
Sample Cell (see *Instrument-specific information*)	1

See *Consumables and replacement items* for reorder information.

Diazotization method for powder pillows

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Fill a sample cell with 10 mL of sample.

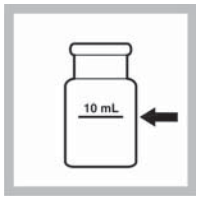
3. **Prepared Sample:** Add the contents of one NitriVer 3 Nitrite Reagent Powder Pillow.

4. Swirl to dissolve.

A pink color will develop if nitrite is present.

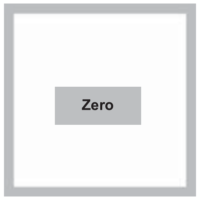
5. Start the instrument timer.

A 20-minute reaction period will begin.



6. **Blank Preparation:** When the timer expires, fill a second sample cell with 10 mL of sample.

7. Wipe the blank and insert it into the cell holder.

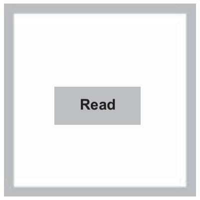


8. **ZERO** the instrument.

The display will show:

0.000 mg/L NO_2^-–N

9. Wipe the prepared sample and insert it into the cell holder.



10. **READ** the results in mg/L NO_2^-–N.

Diazotization method for AccuVac® Ampuls

1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

Refer to the user manual for orientation.

2. Prepared Sample: Collect at least 40 mL of sample into a 50-mL beaker.

Fill a NitriVer 3 Nitrite AccuVac® Ampul with sample. Keep the tip immersed while the Ampul fills.

3. Invert the Ampul several times to mix. A pink color will develop if nitrite is present.

4. Start the instrument timer.

A 20-minute reaction period will begin.

5. Blank Preparation: When the timer expires, fill a sample cell with 10 mL of sample.

6. Wipe the blank and insert it into the cell holder.

ZERO the instrument.

The display will show: 0.000 mg/L NO_2^-–N.

7. Wipe the Ampul and insert it into the cell holder.

READ the results in mg/L NO_2^-–N.

Interferences

Table 2 Interfering substances

Interfering substance	Interference level
Antimonous ions	Interfere by causing precipitation
Auric ions	Interfere by causing precipitation
Bismuth ions	Interfere by causing precipitation
Chloroplatinate ions	Interfere by causing precipitation
Cupric ions	Cause low results
Ferric ions	Interfere by causing precipitation
Ferrous ions	Cause low results
Lead ions	Interfere by causing precipitation
Mercurous ions	Interfere by causing precipitation
Metavanadate ions	Interfere by causing precipitation
Nitrate	Very high levels of nitrate (>100 mg/L nitrate as N) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.
Silver ions	Interfere by causing precipitation
Strong oxidizing and reducing substances	Interferes at all levels

Seawater calibration

Chloride concentrations above 100 mg/L will cause low results. To perform this test in water with high interference level, calibrate the water using standards spiked to the same chloride concentrations as the required samples. To prepare calibration standards containing 0.06, 0.1, 0.3 and 0.4 mg/L nitrate as NO_3–N:

1. Prepare a 1 L volume of chloride water that matches the concentration of the samples, using the following equation:
   - **a.** Add necessary Chloride concentration (g/L) x (1.6485) = g of ACS grade NaCl to 1 L of deionized water. (

     Note: *18.8 g/L is a typical seawater chloride concentration.*
   - **b.** Mix this solution thoroughly to make sure that it is a homogeneous solution. Use this water as the dilution water instead of the deionized water when preparing the nitrate standards.
2. Use Class A glassware or a Tensette Pipet to pipet 0.6, 1, 3 and 4 mL of the 10 mg/L Nitrogen-Nitrate as NO3-N (NIST) Standard Solution (Catalog Number 30749) into four different 100 mL Class A volumetric flasks.
3. Dilute to the mark with the prepared chloride water. Mix thoroughly.
4. Use the prepared chloride water for the 0-mg/L nitrate as NO_3–N standard.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours.
- Warm to room temperature before running the test.
- Do not use acid preservatives.

Accuracy check

Standard solution method

***Note:** Refer to the instrument user manual for specific software navigation instructions.*

1. Preparing nitrite standards is difficult. Use the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, Method 4500—NO_2-B. Prepare a 0.150-mg/L standard.
2. Use the 0.150 mg/L solution in place of the sample. Follow the *Diazotization method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	**OPTIONS>MORE>STANDARD ADJUST**
DR 2800	**OPTIONS>MORE>STANDARD ADJUST**
DR 2700	**OPTIONS>MORE>STANDARD ADJUST**
DR/2500	**OPTIONS>STANDARD ADJUST**
DR/2400	**OPTIONS>STANDARD ADJUST**

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
371	DR 5000	0.150 mg/L NO_2^-–N	0.147–0.153 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N
	DR 2800	0.150 mg/L NO_2^-–N	0.147–0.153 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N
	DR 2700	0.150 mg/L NO_2^-–N	0.147–0.153 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N
	DR/2500	0.150 mg/L NO_2^-–N	0.146–0.154 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N
	DR/2400	0.150 mg/L NO_2^-–N	0.146–0.154 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N
375	DR 5000	0.150 mg/L NO_2^-–N	0.147–0.153 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N
	DR 2800	0.150 mg/L NO_2^-–N	0.147–0.153 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N
	DR 2700	0.150 mg/L NO_2^-–N	0.147–0.153 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N
	DR/2500	0.150 mg/L NO_2^-–N	0.140–0.160 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N
	DR/2400	0.150 mg/L NO_2^-–N	0.140–0.160 mg/L NO_2^-–N	0.002 mg/L NO_2^-–N

Summary of method

Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present. Test results are measured at 507 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
NitriVer® 3 Nitrite Reagent Powder Pillows	1	100/pkg	2107169
OR			
NitriVer® 3 Nitrite Reagent AccuVac® Ampul	1	25/pkg	2512025

Required apparatus (AccuVac)

Description	Quantity/Test	Unit	Catalog number
Beaker, 50-mL	1	each	50041H

Recommended standards, reagents and apparatus

Description	Unit	Catalog number
Balance, Analytical	each	2936701
AccuVac ampules, for blanks	25/pkg	2677925
AccuVac Snapper	each	2405200
AccuVac Drainer	each	4103600
Handbook, Standard Methods for the Examination of Water and Wastewater	each	—
Sodium Nitrite, ACS	454 g	245201
Water, deionized	4 L	27256

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Updated February 2008, Edition 5

Section 18
QA/QC

QA/QC Program

Intermediate Wastewater Treatment
Laboratory

Terms

- A QA/QC program consists of procedures that ensure the precision and accuracy of tests performed on a daily basis.
- Accuracy – estimate on how close a measured value is to the true value; includes expressions for bias and precision
  - Bias – consistent deviation of measured values from the true value, caused by systematic errors in a procedure
  - Precision – a measure of the degree of agreement among replicate analyses of a sample

Quality Assurance

- QA Manual should include the following:
  - Cover sheet with approval signatures
  - Quality policy statement
  - Organizational structure
  - Staff responsibilities
  - Analyst training and performance requirements
  - Tests performed by the lab
  - Procedures for handling and receiving samples
  - Sample control and documentation procedures
  - Procedures for achieving traceable measurements

Quality Assurance

- QA Manual should include the following:
  - Major equipment, instrumentation and reference measurements standards used
  - Standard operating procedures (SOPs) for each analytical method
  - Procedures for generating, approving and controlling policies and procedures
  - Procedures for procuring reference materials and supplies
  - Procedures for procuring subcontractors' services
  - Internal QC activities

Quality Assurance

- QA Manual should include the following:
  - Procedures for calibrating, verifying and maintaining instrumentation and equipment
  - Data-verification practices, including inter-laboratory comparison and proficiency-testing programs
  - Procedures for feedback and corrective actions when testing discrepancies are detected
  - Procedures for permitted exceptions to documented policies
  - Procedures for system and performance audits and reviews
  - Procedures for assessing data precision and accuracy and determining MDLs

Quality Assurance

- QA Manual should include the following:
  - Procedures for data reduction, validation and reporting
  - Procedures for archiving records
  - Procedures and systems for controlling testing environment
  - Procedures for dealing with complaints from data users
  - Also, define the responsibility for and frequency of management review and updates to the QA manual and associated documents

Standard Operating Procedures

- Should contain the following:
  - Title of referenced
  - Consensus test method
  - Sample matrix or matrices
  - MDL
  - Scope and application
  - Summary of SOP
  - Definitions
  - Interferences

Standard Operating Procedures

- Should contain the following:
  - Safety considerations
  - Waste management
  - Apparatus, equipment and supplies
  - Reagents and standards
  - Sample collection, preservation, shipment and storage requirements
  - Specific QC practices, frequency, acceptance criteria and required corrective action if acceptance criteria are not met

Standard Operating Procedures

- Should contain the following:
  - Calibration and standardization
  - Details on the actual test procedure, including sample preparation
  - Calculations
  - Qualifications and performance requirements for analyst
  - Data assessment/data management
  - References
  - Tables, flowcharts and validation or method performance data

Logbooks

- Maintain logbooks for each test or procedure performed, with complete documentation on preparation and analysis of each sample, including:
  - Sample identification
  - Associated standards and QC samples
  - Method reference
  - Date/time of preparation/analysis
  - Analyst
  - Weights and volumes used,
  - Results obtained
  - Any problems encountered
- Keep logbooks that document maintenance and calibration for each instrument or piece of equipment

Record Keeping

- Maintain a complete and accurate list of exact locations of all sampling sites
- Maintain a complete and accurate list of all test procedures used.
  - Record method numbers on bench sheets
- Write in pen
- Initial your entries
- Use a notebook that has numbered pages

Laboratory Conditions

- Lab equipment should be in good working condition
  - Calibrated
  - Checked against standards
- Lab quality distilled water and fresh, pure reagents must be on hand
- Lab temperature should be 68°F

Laboratory Conditions

- Glassware
  - Keep it clean
- pH meter
  - Calibrated daily w/ fresh buffers
- Spectrophotometer

Laboratory Conditions

- Incubator
  - Keep records of temperatures
- Balances
  - Checked at least annually for accuracy
  - Use ASTM Class 1 weights to check calibration

Laboratory Conditions

- Whenever repairs or maintenance are done on lab equipment, record those activities in a log book.

Reagents

- Use only fresh ACS Reagent Grade chemicals
- Check for expiration dates
- Mark chemicals with date arrived in lab and date opened
- When solutions are mixed using dry reagents, record this in a reagent log
  - If standardization is required, include this information in the reagent log as well

Distilled or Deionized Water

- The quality of distilled or deionized water can greatly influence the quality of the test
- There should be no traces of chlorine, ammonia and total suspended solids
- TDS should be less than 1 mg/L
- Distilled is better than deionized
- Ultrapure is better than distilled

Distilled or Deionized Water

- The requirements for reagent grade water spreads across many methods.
- The types are generally type 1 or 2 depending on the analysis.
- A laboratory is required to have the proper type water based on the method and even to the program that the data is being reported.
- In many labs the conductivity indicator is in-line such as a meter or light for deionized water.
- Some stills also have conductivity meter also, if they are in series with another clean-up process

Distilled or Deionized Water

- Where reagent grade water is required, there needs to be some means to assure its quality.
- For most labs that do not have this capability, the use of prepared stock standards and purchased reagent water to make standards dilutions should be used.
- If a still is used the chance of mineral deposit and non-reagent quality water is high.
- Carbonates and ammonia deposition from the air can cause distilled water produced in wastewater plants to be inferior to reagent grade water.

Distilled or Deionized Water

- Either purchase water for critical applications or have a means to assure that the quality of water that is produced in the laboratory is of reagent grade quality.
- If a conductivity meter is used, it is strongly suggested that the standards be purchased to calibrate the meter.

Quality Control Tests

- Duplicates
- Blanks
- Lab Standards
- Unknown Lab Standards
- Spikes

Duplicates

- Simplest form of QC test
- Run two tests on one sample
  - This shows how precise the analyst's procedure is
  - Sample results should yield very close results (goal is to have no difference)
- General recommendation is to run a duplicate sample for every 10 samples

Duplicates – Errors

- Sample size (should be the same size)
- Insufficient mixing
- Dirty glassware
- Calculation errors
- Titration (misreading the burette)
- Weighing
- Calibration
- Reagent water
- Reagents

Duplicates

- Range
  - Difference between the test results
- RPD (Relative Percent Difference)
  - Calculate the average of the two results
  - Divide the range by the average

Duplicates

- You tested a sample for chlorine residual. The sample read 2.1 mg/L and the duplicate sample read 2.2 mg/L. What is the range and RPD?

Range = 2.2 mg/L – 2.1 mg/L = 0.1 mg/L

Average = (2.1+2.2)/2 = 2.15

$$\text{RPD} = \frac{0.1\text{mg/L}}{2.15\text{ mg/L}} = 0.0465 = 4.7\%$$

Blanks

- Blanks can show test interferences
- Blanks should be treated as a sample
  - Take through all procedures
  - Add all reagents or incubate along with other samples
- Target value for blanks is zero

Blanks

- Positive blanks shows problem
  - Bad reagents
  - Bad technique
  - Unclean glassware
  - Bad distilled water

Blanks

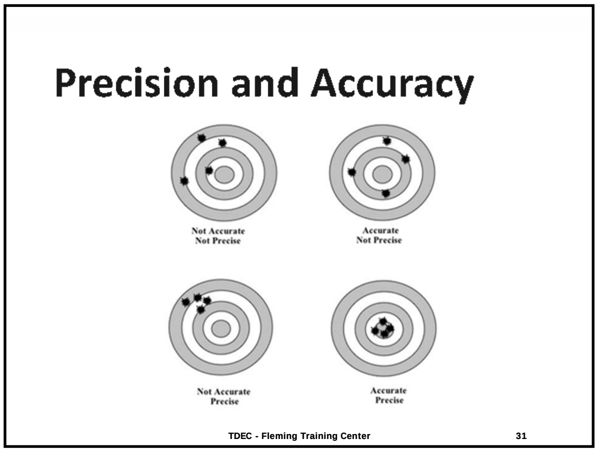
- Fecal or *E. coli* Tests
  - A blank should never be positive
  - If the pre-sample blank has colony growth, the equipment was not properly sterilized
  - If the post sample blank has colony growth, the equipment was not cleaned well enough between samples

Laboratory Standards

- Determines accuracy
- If the test value agrees with the true value, the test has been performed accurately
- Mix onsite or purchased from a supplier
  - Purchased standards should be the preference, because this can reduce the possibility of having mixing errors; they also come with a certificate of analysis
- Perform along with dups., one every 10 samples

Precision and Accuracy

- Accuracy
  - Correctness
  - Checked by using a different method
  - Poor accuracy results from procedural or equipment flaws
- Precision
  - Reproducibility
  - Check by repeating measurements
  - Poor precision results from poor technique



Precision and Accuracy

Unknown Laboratory Samples

- EPA quality control unknowns
- Commercially available
- Gives confidence to analyst
- Can show deficiencies in the testing procedure

Spikes

- Determines accuracy
- A known amount of standard is added to a sample
- The results should equal the sample value plus the added known amount
- Goal is to have 100% recovery of spike and sample
- If your sample result was 100 mg/L and you added 50 mg/L into the spiked sample, you should yield 150 mg/L

Spikes – Hach Example

- Ortho-Phosphate

1. After reading test results, leave the sample cell (unspiked sample) in the instrument. Verify the chemical form.
2. Touch Options. Touch Standard Additions. A summary of the standard additions procedure will appear.
3. Touch OK to accept the default values for standard concentration, sample volume, and spike volumes. Touch Edit to change these values. After values are accepted, the unspiked sample reading will appear in the top row. See Standard Additions in the instrument manual for more information.

Spikes – Hach Example

- Ortho-Phosphate

4. Open a Phosphate 10-mL Ampule Standard, 50-mg/L PO4 3–.
5. Prepare a 0.1-mL sample spike by adding 0.1 mL of standard to the unspiked sample. Touch the timer icon. After the timer beeps, read the result.
6. Prepare a 0.2-mL sample spike by adding 0.1 mL of standard to the 0.1-mL sample spike. Touch the timer icon. After the timer beeps, read the result.

Spikes – Hach Example

- Ortho-Phosphate

7. Prepare a 0.3-mL sample spike by adding 0.1 mL of standard to the 0.2-mL sample spike. Touch the timer icon. After the timer beeps, read the result. Each addition should reflect approximately 100% recovery.
8. After completing the sequence, touch Graph to view the best-fit line through the standard additions data points, accounting for matrix interferences. Touch View: Fit, then select Ideal Line and touch OK to view the relationship between the sample spikes and the “Ideal Line” of 100% recovery.

Split Samples

- Some labs split samples with other labs to check the accuracy of the testing procedure
- If you are concerned that your contracted lab is getting wrong values, send in a known standard as a sample
  - This does double you cost, but you can see how close they are to the known value
  - Don’t tell the contracted lab that the second sample is a known

Physical and Aggregate Properties – Part 2000

Method	Analysis	Calibrate	Method Blank	Duplicates
2540D	TSS		X	X
2540F	Settleable Solids			X
2550B	Temperature	X		

Inorganic Nonmetallic Constituents– Part 4000

Method	Analysis	Method Blank	LFB	LFM and LFMD	Other
4500-Cl G	Total Residual Chlorine - DPD	X	X		2, 3
4500-H^+ B	pH				2, 5
4500-NH_3 D	Ammonia - ISE	X	X	X	3
4500-O G	DO				2, 6

2. Duplicates of the sample will be run
3. Refer to 4020B for further QC requirements
5. Additional QC check with pH sample whose value is bracketed by calibration standards
6. Zero check with zero oxygen sample

Aggregate Organic Constituents– Part 5000

Method	Analysis	Method Blank	LFB	LFM and LFMD	Other
5210 B	5-day BOD test				1, 2, 3

1. Additional QC guidelines in method.
2. Duplicates or LFMD of the sample will be run.
3. Refer to 5020B for further QC requirements.

Wastewater Plant Laboratory Water Information

The quality of water required for use in Wastewater Plant Laboratory varies with use and test method. Reagent water should be free of substances that interfere with analytical methods. The quality of water required is related directly to the analysis being made. Requirements of water quality may differ for organic, inorganic and biological constituents depending on the use for which the water is intended. Any method of preparation of reagent water is acceptable provided the requisite quality can be met.

Standard Methods provides guidelines for reagent water quality in 1080 C. ASTM Standard Specifications for Reagent Water D1193-91 specifies water suitable for use in methods of chemical analysis and physical testing. Storage requirements are discussed in each reference and in the general section of SM 1080 C. The most common tests performed in the wastewater treatment plant laboratory are listed with the references of water quality requirements from Standard Methods.

BOD 5210 A.3. Dilution water requirements. The source of dilution water is not restricted and may be distilled, tap or receiving water free of biodegradable organics and bioinhibitory substances such as chlorine or heavy metals.

Fecal/E. coli 9020 B.3 & 4 preparation and requirements (table 9020II), 9050 B. water specifications

pH 4500-H+ B.3 quality consideration for preparation of in-house buffer solutions (2uS/cm@25^{o}C)

TSS 2540 D. 3 specifies reagent grade water without further detail.

Ammonia 4500-NH_3 B.3.a. use ammonia free distilled water for preparing all reagents, rinsing and sample dilution.

Nitrite 4500 - NO_2^- B.3.a.Use of nitrite free water required for solutions and dilutions.

Nitrate 4500 – NO_3^- B. 3. a. Use of nitrate free water required for solutions and dilutions.

Total Residual Chlorine 4500 – Cl C.3.m. Chlorine-demand –free water quality and preparation

Karina Bynum, P. E.
April 28, 2006
References are from Standard Methods For The Examination of Water and Wastewater, 20th Edition.

ASTM D1193-91
Standard Specification for Reagent Water

	Type I	Type II	Type III	Type IV
Electrical Conductivity uS/cm@25 ^{o}C	0.056	1.0	.25	5.0 max
Electrical Resistivity megohm-cm@25 ^{o}C	18.0	1.0	4.0	0.2 max
pH@25oC	NA	NA	NA	5.0 to8.0
Total Organic Carbon ug/L	50	50	200	No limit
Sodium, max, ug/L	1	5	10	50
Chlorides, max, ug/L	1	5	10	50
Total Silica, max, ug/L	3	3	500	No limit

SM 1080 C
Reagent Water Quality

	High	Medium	Low
Electrical Conductivity uS/cm@25 ^{o}C	<0.1	<1	10
Electrical Resistivity megohm-cm@25 ^{o}C	>10	>1	0.1
SiO_2 mg/L	<0.05	<0.1	<1

SM 9020
Quality of Reagent Water Used in Microbiology Testing

	Limit
Conductivity uS/cm@25oC	<2
pH standard units	5.5-7.5
Total Organic Carbon mg/L	<1.0
Heavy metals, single (Cd, Cr, Cu, Ni, Pb, Zn) mg/L	<0.05
Heavy metals total mg/L	<0.10
Ammonia/organic nitrogen mg/L	<0.10
Total Chlorine Residual mg/L	<0.01

Karina Bynum, P. E.
April 28, 2006
References are from Standard Methods For The Examination of Water and Wastewater, 20th Edition.

Section 19
Instrument and Probe Maintenance

Instrument and Probe Maintenance

Wastewater Treatment Lab

Instruments

- Whenever you have any maintenance done on an instrument, you should keep records on it
  - After maintenance, you should check calibration
- Keep records on calibrations

Instruments

- Good idea to have separate book for each instrument
- Keep instruments clean so they work best
- Always have replacement parts on hand
  - Bulbs for turbidimeters and spectrophotometers

pH Meters

- Good recommendation: water industry should not use gel filled probes
- Calibrate DAILY with fresh buffers
- Three buffers are better than two
  - If you use two buffers, you should use buffers that bracket your normal readings
  - 10 buffer degrades faster than 4 and 7

pH Probes

- Store probes in slightly acidic solution
  - Can be bought premixed
  - pH 4 buffer works also
- Don't store in distilled water or finished water
  - Becomes slow to respond
- pH electrode (glass ball) is porous for H^+ to migrate through
- Can clean with dilute hydrochloric acid (HCl) is slow response for 30-60 seconds then rinse with distilled water for 15-20 minutes
  - Follow manufacturer's recommendation

pH Probes

- Replace annually
  - Good practice to put date on probe
  - Have back up probe at all times
- For fillable probes, if crystals form in reference electrode area
  - Shake out solution
  - Fill with distilled water until crystals dissolve
  - Dump out then refill with correct solution

Section 20
Oil and Grease

Oil & Grease

Liquid/Liquid
OR
Solid Phase

Currently Approved Methods

- EPA Method 1664A
- SM 5520B (20th edition only)
  - Solvent type limited to hexane only

Sample Collection/Preservation

- Glass wide mouth container (SM 5520A.3)
- Clean sample bottles with solvent
- Grab sample
- Use PTFE-lined caps or line cap with foil
- Collect (3)1 liter of samples (EPA 1664A)
- Acidify to pH < 2 with H_2SO_4 or HCL
- Cool to ≤6°C and analyze within 28 days

Summary of Method

- Two basic techniques used are:

1. Liquid/Liquid extraction for partitioning followed by gravimetric determinations.
2. Solid phase extraction followed by elution and then gravimetric determinations.

Significance of O & G

- If present in excessive amount, O&G may interfere with aerobic and anaerobic biological processes and lead to decreased wastewater treatment efficiency.
- Excess amounts of O&G if discharged may cause surface films and shoreline deposits leading to environmental degradation.

SOURCES OF O&G

- Animal fat
  - e.g. meat packaging plant & fish processing
- Vegetable Oils
  - e.g. food processing & restaurants
- Petroleum Products
  - e.g. refinery & asphalt

EPA METHOD 1664A

- Hexane extractable materials (HEM)
- Silica-Gel treatment (SGT-HEM) for petroleum products
- Measurements of materials that volatilize approximately below 85°C

Calibration & Standardization

- Calibrate the analytical balance at 2 mg and 100 mg using class "ASTM Class 1" weights
- Calibration shall be within ± 10% at 2 mg and ± 0.5% at 1000 mg. If values are not within these limits, the balance must be recalibrated

Liquid/Liquid Extraction Procedure

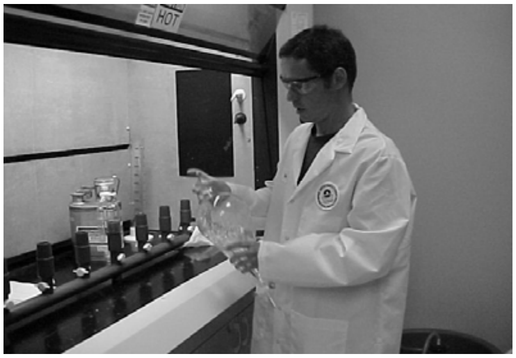
- Volume measurements
  - Mark sample bottle
  - Weigh sample and bottle
- Check pH of sample (<2) with stirring rod
- Transfer sample to a 2L separatory funnel
- Vigorous extraction for 2 minutes each with 30 mL hexane
- Extract sample three times; drain solvent with each extraction

Liquid/Liquid Extraction Procedure (continued)

- Filter each extraction through a funnel containing 10g of Na_2SO_4, rinse with hexane and collect into a pre-weighed boiling flask
- Distill solvent at approximately 70°C at the distilling head
- Sweep out the flask for 15 seconds using a vacuum to remove any remaining solvent vapors

Liquid/Liquid Extraction Procedure (continued)

- Desiccate boiling flask containing residue for at least 30 minutes
- Weigh boiling flask and residue until measurement reaches a constant weight; within 4% or less than 0.5 mg whichever is less.

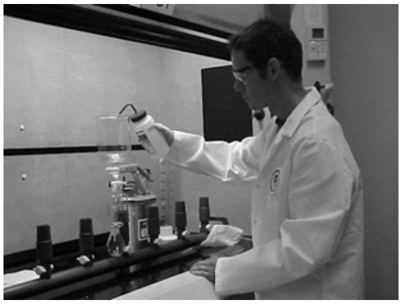


Solid Phase Extraction Procedure

- Volume measurements
  - Mark sample bottle
  - Weigh sample and bottle
- Check pH of sample (<2) with stirring rod
- Filter sample through solid phase disks
- Rinse sample bottle with 10 mL of hexane and pour over filter disks to elute sample
- Repeat this process 2 to 3 times with the 10 mL volumes of hexane

Solid Phase Extraction Procedure (continued)

- Collect solvent into a pre-weighed container
- Heat solvent at approximately 70°C and recover the condensate/distillate
- Sweep out the container for 15 seconds using a vacuum to remove any remaining solvent vapors
- Cool sample and dessicate prior to weighing
- Dessicate for 30 minutes and obtain constant weight



Calculations

$$Mg/L = \frac{final\ wt - initial\ weight(mg)\ x\,1000}{volume\ of\ \ sample\ (mL)}$$

Quality Control

- Initial demonstration of ability for accuracy and precision.
- Method Detection Limit (MDL) Study
- Blanks
- Method acceptance criteria found in Table I
  - Laboratory Control Sample (stearic acid & hexadecane)
  - Quality Control Sample (2nd Source)
  - Spikes
  - Matrix Spikes

Waste Management

- It's the facilities responsibility to make sure the waste is recycled or disposed of properly

Section 21

Activated Sludge Math

Applied Math for Wastewater Treatment
Activated Sludge

BOD or COD Loading, lbs/day

- This is the food part of the F/M ratio
- COD is sometimes used if there is a good correlation between it and BOD
- Loading guidelines for the 3 operational modes of Activated Sludge are:
  - High Rate
    - COD: greater than 1 lb COD/day/lb MLVSS under aeration
    - BOD: greater than 0.5 lb BOD/day/lb MLVSS under aeration
  - Conventional
    - COD: 0.5 to 1 lb COD/day/lb MLVSS under aeration
    - BOD: 0.25 to 0.5 lb BOD/day/lb MLVSS under aeration
  - Extended Aeration
    - COD: less than 0.2 lb COD/day/lb MLVSS under aeration
    - BOD: less than 0.1 lb BOD/day/lb MLVSS under aeration
- For untreated domestic wastewater, BOD = (0.4 to 0.8)(COD)

Solids Inventory in the Aeration Tank, lbs. MLSS or lbs. MLVSS

- In an activated sludge system, the solids under aeration must be controlled
- The SS in aeration tank are the MLSS
- MLVSS is an estimate of the microorganism population in the aeration tank.
- The MLVSS is typically 70% of the MLSS, the remaining 30% are fixed (or inorganic) solids

Food to Microorganism Ratio

- In order for an Activated Sludge system to operate properly, there must be a balance between the food (BOD or COD) and bugs in the aeration tank (MLVSS).
- The F/M ratio is a process control calculation used in many activated sludge plants
- Best F/M depends on the type of activated sludge system and the wastewater characteristics
- The F/M ratio is calculated from the amount of BOD or COD applied each day and from the solids inventory in the aeration tank.
- Typical ranges for F/M (using BOD):
  - Conventional ranges are 0.2-0.4
  - Extended Aeration ranges are 0.05-0.15

Mean Cell Residence Time (MCRT), days

- Also called SRT, Solids Retention Time
- Approach used for solids control, adjust WAS to maintain MCRT
- Most desirable MCRT for a plant is determined experimentally
- Typical ranges are:
    - Conventional plants MCRT is 5-15 days
    - Extended aeration MCRT is 20-30 days
- MCRT based on suspended solids leaving the system and includes the aeration tank and final clarifier
- Also can determine the type of bugs that predominate and therefore the degree of nitrification that may occur
    - From AWT Table 2.6: MCRT needed to produce nitrified effluent as related to temp
        - 10ºC – 30 days
        - 15ºC – 20 days
        - 20ºC – 15 days
        - 25ºC – 10 days
        - 30ºC – 7 days

Wasting Rates

- The amount of activated sludge wasted may vary from 1-20% of total incoming flow
- Expressed in lbs or gallons/day
- Wasting is the diverting of flow to primary clarifier, thickener, gravity belt thickener or aerobic or anaerobic digester

Applied Math for Wastewater Treatment
Activated Sludge

BOD or COD Loading, lbs/day

1. The flow to an aeration tank is 850,000 gpd. If the BOD content of the wastewater entering the aeration tank is 225 mg/L, how many pounds of BOD are applied to the aeration tank daily?

2. The flow to an aeration tank is 1200 gpm. If the COD concentration of the wastewater is 155 mg/L, what is the COD loading rate in lbs/day?

Solids Inventory in the Aeration Tank, lbs. MLSS or lbs. MLVSS

3. An aeration basin is 120 ft long, 45 ft wide and holds wastewater to a depth of 12 ft. If the aeration basin has an MLSS concentration of 2150 mg/L, how many pounds of MLSS are under aeration?

4. The aeration tank of a conventional activated sludge plant has an MLSS concentration of 2300 mg/L with a volatile solids content of 72%. If the volume of the aeration tank is 200,000 gallons, how many pounds of volatile solids are under aeration?

Food to Microorganism Ratio

5. An activated sludge aeration tank receives a primary effluent flow of 1.6 MGD with a BOD concentration of 180 mg/L. The mixed liquor volatile suspended solids is 2200 mg/L and the aeration tank volume is 420,000 gallons. What is the current F/M ratio?

6. The flow to a 195,000 gallon oxidation ditch is 365,000 gpd. The BOD concentration of the wastewater is 170 mg/L. If the MLSS concentration is 2550 mg/L with a volatile content of 70%, what is the F/M ratio?

7. The desired F/M ratio of an extended aeration activated sludge plant is 0.5 lbs COD/lb. MLVSS. If the 3.0 MGD primary effluent flow has a COD of 172 mg/L, how many lbs of MLVSS should be maintained in the aeration tank?

Mean Cell Residence Time (MCRT), days

8. An activated sludge system has a total of 28,500 lbs of mixed liquor suspended solids. The suspended solids leaving the final clarifier in the effluent is 400 lbs/day. The pounds suspended solids wasted from the final clarifier is 2910 lbs/day. What is the solids retention time (MCRT), days?

9. Determine MCRT given the following information:

Aeration Tank = 1,400,000 gal
Final Clarifier = 105,000 gal
Flow = 3,000,000 gpd
WAS Pump Rate = 68,000 gpd

MLSS = 2650 mg/L
S.E. SS = 22 mg/L
CCSS = 1890 mg/L
WAS = 6050 mg/L

Wasting Rates

10. Using Constant F/M Ratio: The desired F/M ratio for an activated sludge system is 0.6 lbs BOD/lb MLVSS. It has been calculated that 3300 lbs of BOD enter the aeration basin daily. If the volatile solids content of the MLSS is 68%, how many lbs MLSS are desired in the aeration basin?

11. Using Constant MCRT: The desired MCRT for an activated sludge plant is 8.5 days. The secondary effluent flow is 3.16 MGD with a suspended solids content of 22 mg/L. There is a total of 32,100 lbs SS in the system. How many lbs/day WAS SS must be wasted to maintain the desired MCRT?

Answers:

1. 1595 lbs BOD/day
2. 2234 lbs COD/day
3. 8691 lbs MLSS
4. 2762 lbs MLVSS
5. 0.31
6. 0.18
7. 8607 lbs MLVSS
8. 8.6 days
9. 8.2 days
10. 8088 lbs MLSS desired
11. 3197 lbs to waste

Applied Math for Wastewater Treatment
Activated Sludge
Extra Problems

BOD or COD Loading, lbs/day

1. The flow to an aeration basin is 880,000 gpd. If the BOD content of the wastewater entering the aeration basin is 240 mg/L, what is the lbs/day BOD loading?

2. The flow to the aeration basin is 2980 gpm. If the COD concentration of the wastewater is 160 mg/L, how many lbs of COD are applied to the aeration basin daily?

3. The BOD content of the wastewater entering an aeration basin is 165 mg/L. If the flow to the aeration basin is 3,240,000 gpd, what is the lbs/day BOD loading?

4. The daily flow to an aeration basin is 4,880,000 gpd. If the COD concentration of the influent wastewater is 150 mg/L, how many lbs of COD are applied to the aeration basin daily?

Solids Inventory in the Aeration Basin, lbs. MLSS or lbs. MLVSS

5. If the mixed liquor suspended solids concentration is 2110 mg/L and the aeration basin has a volume of 460,000 gallons, how many lbs of suspended solids are in the aeration basin?

6. The aeration basin of a conventional activated sludge plant has a mixed liquor volatile suspended solids (MLVSS) concentration of 2420 mg/L. If the aeration basin is 90 ft long by 50 ft wide and has wastewater to a depth of 16 ft, how many lbs of MLVSS are under aeration?

7. The aeration basin of a conventional activated sludge plant has a mixed liquor volatile suspended solids (MLVSS) concentration of 2410 mg/L. If the aeration basin is 80 ft long by 40 ft wide and has wastewater to a depth of 16 ft, how many lbs of MLVSS are under aeration?

8. An aeration basin is 110 ft long, 30 ft wide and has wastewater to a depth of 16 ft. If the aeration basin of this conventional activated sludge plant has a mixed liquor suspended solids (MLSS) concentration of 2740 mg/L, how many lbs of MLSS are under aeration?

9. An aeration basin is 110 ft long, 50 ft wide and has wastewater to a depth of 16 ft. If the mixed liquor suspended solids (MLSS) concentration in the aeration basin is 2470 mg/L with a volatile solids content of 73%, how many lbs of MLVSS are under aeration?

Food to Microorganism Ratio

10. An activated sludge aeration basin receives a primary effluent flow of 2.72 MGD with a BOD concentration of 198 mg/L. The mixed liquor volatile suspended solids (MLVSS) concentration is 2610 mg/L and the aeration basin volume is 480,000 gallons. What is the current F/M ratio?

11. An activated sludge aeration basin receives a primary effluent flow of 3,350,000 gpd with a BOD of 148 mg/L. The mixed liquor volatile suspended solids (MLVSS) concentration is 2510 mg/L and the aeration basin volume is 490,000 gallons. What is the F/M ratio?

12. The flow to a 195,000 gallon oxidation ditch is 320,000 gpd. The BOD concentration of the wastewater is 180 mg/L. If the mixed liquor suspended solids (MLSS) concentration is 2540 mg/L with a volatile solids content of 72%, what is the F/M ratio?

13. The desired F/M ratio at an extended aeration activated sludge plant is 0.7 lb BOD/lb MLVSS. If the primary effluent flow is 3.3 MGD and has a BOD of 181 mg/L, how many pounds of MLVSS should be maintained in the aeration basin?

14. The desired F/M ratio at a particular activated sludge plant is 0.4 lbs BOD/lb MLVSS. If the primary effluent flow is 2,510,000 gpd and has a BOD concentration of 141 mg/L, how many lbs of MLVSS should be maintained in the aeration basin?

Mean Cell Residence Time (MCRT), days

15. An activated sludge system has a total of 29,100 lbs of MLSS. The concentration of suspended solids leaving the final clarifier in the effluent is calculated to be 400 lbs/day. Suspended solids wasted from the clarifier are 2920 lbs/day. What is the MCRT in days?

16. Determine the MCRT given the following data: aeration basin volume, 1,500,000 gallons; mixed liquor suspended solids, 2710 mg/L; final clarifier, 106,000 gallons; waste activated sludge, 5870 mg/L; WAS pumping rate, 72,000 gpd; plant flow, 3.3 MGD; secondary effluent SS, 25 mg/L; average clarifier core SS, 1940 mg/L.

17. An aeration basin has a volume of 460,000 gallons. The final clarifier has a volume of 178,000 gallons. The MLSS concentration in the aeration basin is 2222 mg/L. If 1610 lbs/day suspended solids are wasted and 240 lbs/day suspended solids are in the secondary effluent, what is the MCRT for the activated sludge system?

18. Determine MCRT given the following information:

Aeration Basin = 350,000 gal
Final Clarifier = 125,000 gal
Flow = 1,400,000 gpd
WAS Pump Rate = 27,000 gpd

MLSS = 2910 mg/L
S.E. SS = 16 mg/L
WAS = 6210 mg/L

Wasting Rates

19. Using Constant F/M Ratio: The desired F/M ratio for an activated sludge system is 0.5 lbs BOD/lb MLVSS. It has been calculated that 3400 lbs of BOD enter the aeration basin daily. If the volatile solids content of the MLSS is 69%, how many lbs MLSS are desired in the aeration basin?

20. Using Constant MCRT: The desired MCRT for an activated sludge plant is 9 days. The secondary effluent flow is 3,220,000 gpd with a suspended solids content of 23 mg/L. There is a total of 32,400 lbs SS in the system. How many lbs/day WAS SS must be wasted to maintain the desired MCRT?

21. Given the following data, determine the lbs/day suspended solids to be wasted:

Aeration Tank Volume = 1.2 MG
Influent Flow = 3,100,000 gpd
BOD = 110 mg/L

Desired F/M = 0.4
MLSS = 2200 mg/L
%VS = 68%

Answers:

1. 1761 lbs BOD/day
2. 5726 lbs COD/day
3. 4459 lbs BOD/day
4. 6105 lbs COD/day
5. 8095 lbs MLSS
6. 10,870 lbs MLVSS
7. 7698 lbs MLVSS
8. 9025 lbs MLSS
9. 9899 lbs MLVSS
10. 0.43
11. 0.40
12. 0.16
13. 7116 lbs MLVSS
14. 7379 lbs MLVSS
15. 8.8 days
16. 8.5 days
17. 6.4 days
18. 7.3 days
19. 9855 lbs MLSS desired
20. 2982 lbs MLSS to waste
21. 11,562 lbs MLSS to waste

Applied Math for Wastewater Treatment
Activated Sludge

BOD or COD Loading, lbs/day

pg. 10 in formula book

1. The flow to an aeration tank is 850,000 gpd. If the BOD content of the wastewater entering the aeration tank is 225 mg/L, how many pounds of BOD are applied to the aeration tank daily?

$$\text{lbs BOD/day} = (\text{BOD, mg/L})(\text{Flow, MGD})(8.34)$$
$$= (225\ \text{mg/L})(0.85\ \text{MG})(8.34)$$
$$= \boxed{1595\ \text{lbs/d}}$$

2. The flow to an aeration tank is 1200 gpm. If the COD concentration of the wastewater is 155 mg/L, what is the COD loading rate in lbs/day?

$$\frac{(1200\ \text{gpm})(1440)}{1{,}000{,}000} = 1.728\ \text{MGD}$$

$$\text{lbs COD/day} = (\text{COD, mg/L})(\text{Flow, MGD})(8.34)$$
$$= (155\ \text{mg/L})(1.728\ \text{MGD})(8.34)$$
$$= \boxed{2234\ \text{lbs/d}}$$

Solids Inventory in the Aeration Tank, lbs. MLSS or lbs. MLVSS

3. An aeration basin is 120 ft long, 45 ft wide and holds wastewater to a depth of 12 ft. If the aeration basin has an MLSS concentration of 2150 mg/L, how many pounds of MLSS are under aeration?

$$\text{aerator volume, gal} = (120\ \text{ft})(45\ \text{ft})(12\ \text{ft})(7.48) = 484{,}704\ \text{gal}$$

$$\text{MLSS, lbs} = (\text{MLSS, mg/L})(\text{Aer. Vol, MG})(8.34)$$
$$= (2150\ \text{mg/L})(0.484704\ \text{MG})(8.34)$$
$$= \boxed{8691\ \text{lbs MLSS}}$$

4. The aeration tank of a conventional activated sludge plant has an MLSS concentration of 2300 mg/L with a volatile solids content of 72%. If the volume of the aeration tank is 200,000 gallons, how many pounds of volatile solids are under aeration?

$$\text{MLVSS, lbs} = (\text{MLSS, mg/L})(\text{Aer. Vol., MG})(8.34)(\%\text{VS})$$
$$= (2300\text{ mg/L})(0.2\text{ MG})(8.34)(0.72)$$
$$= \boxed{2762\text{ lbs MLVSS}}$$

Food to Microorganism Ratio

5. An activated sludge aeration tank receives a primary effluent flow of 1.6 MGD with a BOD concentration of 180 mg/L. The mixed liquor volatile suspended solids is 2200 mg/L and the aeration tank volume is 420,000 gallons. What is the current F/M ratio?

$$F/M = \frac{(\text{BOD or COD, mg/L})(\text{Flow, MGD})(8.34)}{(\text{MLVSS, mg/L})(\text{Aer. Vol., MG})(8.34)}$$
$$= \frac{(180\text{ mg/L})(1.6\text{ MGD})(8.34)}{(2200\text{ mg/L})(0.42\text{ MG})(8.34)} = \frac{2401.92}{7706.16} = \boxed{0.31}$$

6. The flow to a 195,000 gallon oxidation ditch is 365,000 gpd. The BOD concentration of the wastewater is 170 mg/L. If the MLSS concentration is 2550 mg/L with a volatile content of 70%, what is the F/M ratio?

$$F/M = \frac{(170\text{ mg/L})(0.365\text{ MGD})(8.34)}{(2550\text{ mg/L})(0.195\text{ MG})(8.34)(0.70)}$$
$$= \frac{517.497}{2902.9455} = \boxed{0.18}$$

7. The desired F/M ratio of an extended aeration activated sludge plant is 0.5 lbs COD/lb. MLVSS. If the 3.0 MGD primary effluent flow has a COD of 172 mg/L, how many lbs of MLVSS should be maintained in the aeration tank?

$$\text{Desired MLVSS, lbs} = \frac{\text{BOD or COD, lbs}}{\text{Desired F/M ratio}}$$
$$= \frac{(172\text{ mg/L})(3.0\text{ MGD})(8.34)}{0.5}$$
$$= \boxed{8607\text{ lbs}}$$

Mean Cell Residence Time (MCRT), days pg. 11 formula book

8. An activated sludge system has a total of 28,500 lbs of mixed liquor suspended solids. The suspended solids leaving the final clarifier in the effluent is 400 lbs/day. The pounds suspended solids wasted from the final clarifier is 2910 lbs/day. What is the solids retention time (MCRT), days?

$$\text{MCRT} = \frac{\text{SS in System, lbs}}{\text{WAS, lbs/d} + \text{SE SS lbs/d}} = \frac{28{,}500 \text{ lbs}}{2910 \text{ lbs/d} + 400 \text{ lbs/d}}$$

$$= \frac{28{,}500 \text{ lbs}}{3310 \text{ lbs/d}}$$

$$= \boxed{8.6 \text{ days}}$$

9. Determine MCRT given the following information:

Aeration Tank = 1,400,000 gal
Final Clarifier = 105,000 gal
Flow = 3,000,000 gpd
WAS Pump Rate = 68,000 gpd

MLSS = 2650 mg/L
S.E. SS = 22 mg/L
CCSS = 1890 mg/L
WAS = 6050 mg/L

$$\text{MCRT, days} = \frac{(\text{MLSS, mg/L})(\text{Aer. Vol, MG})(8.34) + (\text{CCSS, mg/L})(\text{Final Clar., Vol})(8.34)}{(\text{WAS, SS mg/L})(\text{WAS Flow MGD})(8.34) + (\text{SE SS mg/L})(\text{Plant Flow})(8.34)}$$

$$= \frac{(2650)(1.4)(8.34) + (1890)(0.105)(8.34)}{(6050)(0.068)(8.34) + (22)(3.0)(8.34)}$$

$$= \frac{30941.4 + 1655.073}{3431.076 + 550.44} = \frac{32596.473}{3981.516} = \boxed{8.2 \text{ days}}$$

Wasting Rates

10. Using Constant F/M Ratio: The desired F/M ratio for an activated sludge system is 0.6 lbs BOD/lb MLVSS. It has been calculated that 3300 lbs of BOD enter the aeration basin daily. If the volatile solids content of the MLSS is 68%, how many lbs MLSS are desired in the aeration basin?

$$\text{Desired MLVSS, lbs} = \frac{3300 \text{ lbs BOD}}{0.6} = 5500 \text{ lbs}$$

$$\text{Desired MLSS, lbs} = \frac{5500 \text{ lbs MLVSS}}{0.68} = \boxed{8088 \text{ lbs}}$$

11. Using Constant MCRT: The desired MCRT for an activated sludge plant is 8.5 days. The secondary effluent flow is 3.16 MGD with a suspended solids content of 22 mg/L. There is a total of 32,100 lbs SS in the system. How many lbs/day WAS SS must be wasted to maintain the desired MCRT?

$$\text{WAS, lbs/d} = \frac{(\text{MLSS, mg/L})(\text{Aer. Vol, MG})(8.34)}{\text{MCRT, days}} - (\text{SE SS, mg/L})(\text{Plant Q})(8.34)$$

$$= \frac{32{,}100\ \text{lbs}}{8.5\ \text{days}} - (22\ \text{mg/L})(3.16\ \text{MGD})(8.34)$$

$$= 3776.4706\ \text{lbs/d} - 579.7968\ \text{lbs/d}$$

$$= \boxed{3197\ \text{lbs/d}}$$

Answers:

1. 1595 lbs BOD/day
2. 2234 lbs COD/day
3. 8691 lbs MLSS
4. 2762 lbs MLVSS
5. 0.31
6. 0.18
7. 8607 lbs MLVSS
8. 8.6 days
9. 8.2 days
10. 8088 lbs MLSS desired
11. 3197 lbs to waste

Applied Math for Wastewater Treatment
Activated Sludge
Extra Problems

BOD or COD Loading, lbs/day

1. The flow to an aeration basin is 880,000 gpd. If the BOD content of the wastewater entering the aeration basin is 240 mg/L, what is the lbs/day BOD loading?

$$\text{lbs/d} = (240 \text{ mg/L})(0.88 \text{ MGD})(8.34)$$

$$= \boxed{1761 \text{ lbs/d}}$$

2. The flow to the aeration basin is 2980 gpm. If the COD concentration of the wastewater is 160 mg/L, how many lbs of COD are applied to the aeration basin daily?

$$\frac{(2980)(1440)}{1{,}000{,}000} = 4.2912 \text{ MGD}$$

$$\text{lbs/d} = (160 \text{ mg/L})(4.2912 \text{ MGD})(8.34) = \boxed{5726 \text{ lbs/d}}$$

3. The BOD content of the wastewater entering an aeration basin is 165 mg/L. If the flow to the aeration basin is 3,240,000 gpd, what is the lbs/day BOD loading?

$$\text{lbs/d} = (165 \text{ mg/L})(3.24 \text{ MGD})(8.34)$$

$$= \boxed{4459 \text{ lbs/d}}$$

4. The daily flow to an aeration basin is 4,880,000 gpd. If the COD concentration of the influent wastewater is 150 mg/L, how many lbs of COD are applied to the aeration basin daily?

$$\text{lbs/d} = (150 \text{ mg/L})(4.88 \text{ MGD})(8.34)$$

$$= \boxed{6105 \text{ lbs/d}}$$

Solids Inventory in the Aeration Basin, lbs. MLSS or lbs. MLVSS

5. If the mixed liquor suspended solids concentration is 2110 mg/L and the aeration basin has a volume of 460,000 gallons, how many lbs of suspended solids are in the aeration basin?

$$\text{lbs} = (2110\text{ lbs})(0.46\text{ MG})(8.34)$$

$$= \boxed{8095\text{ lbs}}$$

6. The aeration basin of a conventional activated sludge plant has a mixed liquor volatile suspended solids (MLVSS) concentration of 2420 mg/L. If the aeration basin is 90 ft long by 50 ft wide and has wastewater to a depth of 16 ft, how many lbs of MLVSS are under aeration?

$$\text{vol.} = \frac{(90\text{ft})(50\text{ft})(16\text{ft})(7.48)}{1{,}000{,}000} = 0.53856\text{ MG}$$

$$\text{lbs} = (2420\text{ mg/L})(0.53856\text{ MG})(8.34) = \boxed{10{,}870\text{ lbs}}$$

7. The aeration basin of a conventional activated sludge plant has a mixed liquor volatile suspended solids (MLVSS) concentration of 2410 mg/L. If the aeration basin is 80 ft long by 40 ft wide and has wastewater to a depth of 16 ft, how many lbs of MLVSS are under aeration?

$$\text{vol.} = \frac{(80\text{ft})(40\text{ft})(16\text{ft})(7.48)}{1{,}000{,}000} = 0.382976\text{ MG}$$

$$\text{lbs} = (2410\text{ mg/L})(0.382976\text{ MG})(8.34) = \boxed{7698\text{ lbs}}$$

8. An aeration basin is 110 ft long, 30 ft wide and has wastewater to a depth of 16 ft. If the aeration basin of this conventional activated sludge plant has a mixed liquor suspended solids (MLSS) concentration of 2740 mg/L, how many lbs of MLSS are under aeration?

$$\text{vol.} = \frac{(110\text{ft})(30\text{ft})(16\text{ft})(7.48)}{1{,}000{,}000} = 0.394944\text{ lbs}$$

$$\text{lbs} = (2740\text{ mg/L})(0.394944\text{ lbs})(8.34) = \boxed{9025\text{ lbs}}$$

9. An aeration basin is 110 ft long, 50 ft wide and has wastewater to a depth of 16 ft. If the mixed liquor suspended solids (MLSS) concentration in the aeration basin is 2470 mg/L with a volatile solids content of 73%, how many lbs of MLVSS are under aeration?

$$\text{Vol} = \frac{(110\text{ ft})(50\text{ ft})(16\text{ ft})(7.48)}{1{,}000{,}000} = 0.65824\text{ MG}$$

$$\text{lbs} = \underbrace{(2470\text{ mg|L})(0.73)}_{\text{gives you mg|L MLVSS}}(0.65824)(8.34) = \boxed{9899\text{ lbs}}$$

Food to Microorganism Ratio

10. An activated sludge aeration basin receives a primary effluent flow of 2.72 MGD with a BOD concentration of 198 mg/L. The mixed liquor volatile suspended solids (MLVSS) concentration is 2610 mg/L and the aeration basin volume is 480,000 gallons. What is the current F/M ratio?

$$\text{F|M} = \frac{(198\text{ mg|L})(2.72\text{ MGD})(8.34)}{(2610\text{ mg|L})(0.48\text{ MG})(8.34)}$$

$$= \frac{4491.5904\text{ lbs|d BOD}}{10448.352\text{ lbs MLVSS}} = \boxed{0.43}$$

11. An activated sludge aeration basin receives a primary effluent flow of 3,350,000 gpd with a BOD of 148 mg/L. The mixed liquor volatile suspended solids (MLVSS) concentration is 2510 mg/L and the aeration basin volume is 490,000 gallons. What is the F/M ratio?

$$\text{F|M} = \frac{(148\text{ mg|L})(3.35\text{ MGD})(8.34)}{(2510\text{ mg|L})(0.49\text{ MG})(8.34)}$$

$$= \frac{4134.972\text{ lbs|d BOD}}{10257.366\text{ lbs MLVSS}} = \boxed{0.40}$$

12. The flow to a 195,000 gallon oxidation ditch is 320,000 gpd. The BOD concentration of the wastewater is 180 mg/L. If the mixed liquor suspended solids (MLSS) concentration is 2540 mg/L with a volatile solids content of 72%, what is the F/M ratio?

$$F/M = \frac{(180\text{ mg/L})(0.32\text{ MGD})(8.34)}{(2540\text{ mg/L})(0.72)(0.195\text{ MG})(8.34)}$$

$$= \frac{480.384\text{ lbs/d BOD}}{2974.17744\text{ lbs MLVSS}} = \boxed{0.16}$$

13. The desired F/M ratio at an extended aeration activated sludge plant is 0.7 lb BOD/lb MLVSS. If the primary effluent flow is 3.3 MGD and has a BOD of 181 mg/L, how many pounds of MLVSS should be maintained in the aeration basin?

$$\text{Desired MLVSS, lbs} = \frac{(181\text{ mg/L})(3.3\text{ MGD})(8.34)}{0.7}$$

$$= \boxed{7116\text{ lbs MLVSS}}$$

14. The desired F/M ratio at a particular activated sludge plant is 0.4 lbs BOD/lb MLVSS. If the primary effluent flow is 2,510,000 gpd and has a BOD concentration of 141 mg/L, how many lbs of MLVSS should be maintained in the aeration basin?

$$\text{Desired MLVSS, lbs} = \frac{(141\text{ mg/L})(2.51\text{ MGD})(8.34)}{0.4}$$

$$= \boxed{7379\text{ MLVSS lbs}}$$

Mean Cell Residence Time (MCRT), days

in system

15. An activated sludge system has a total of 29,100 lbs of MLSS. The concentration of suspended solids leaving the final clarifier in the effluent is calculated to be 400 lbs/day. Suspended solids wasted from the clarifier are 2920 lbs/day. What is the MCRT in days?

leaving

$$\text{MCRT, days} = \frac{29{,}100\text{ lbs}}{400\text{ lbs/d} + 2920\text{ lbs/d}}$$

$$= \frac{29{,}100\text{ lbs}}{3320\text{ lbs/d}} = \boxed{8.8\text{ days}}$$

16. Determine the MCRT given the following data: aeration basin volume, 1,500,000 gallons; mixed liquor suspended solids, 2710 mg/L; final clarifier, 106,000 gallons; waste activated sludge, 5870 mg/L; WAS pumping rate, 72,000 gpd; plant flow, 3.3 MGD; secondary effluent SS, 25 mg/L; average clarifier core SS, 1940 mg/L.

Aer. Basin = 1,500,000 gal
Clarifier = 106,000 gal
WAS Pump = 72,000 gpd
Plant Flow = 3.3 MGD

MLSS = 2710 mg/L
WAS = 5870 mg/L
SE SS = 25 mg/L
CC SS = 1940 mg/L

★ Use 5th MCRT Formula ★

$$\text{MCRT} = \frac{(2710)(1.5)(8.34) + (1940)(0.106)(8.34)}{(5870)(0.072)(8.34) + (25)(3.3)(8.34)}$$

$$= \frac{33{,}902.1 + 1715.0376}{3524.8176 + 688.05} = \frac{35617.1376}{4212.8676} = \boxed{8.5 \text{ days}}$$

17. An aeration basin has a volume of 460,000 gallons. The final clarifier has a volume of 178,000 gallons. The MLSS concentration in the aeration basin is 2222 mg/L. If 1610 lbs/day suspended solids are wasted and 240 lbs/day suspended solids are in the secondary effluent, what is the MCRT for the activated sludge system?

Aer. Basin = 460,000 gal
Clarifier = 178,000 gal
MLSS = 2222 mg/L

SS wasted = 1610 lbs/d
SE SS = 240 lbs/d

★ Use top of 4th MCRT formula & bottom of 2nd formula ★

$$\text{MCRT, days} = \frac{(\text{MLSS, mg/L})(\text{Aer. Vol} + \text{Clarifier Vol., MG})(8.34)}{\text{WAS, lbs/d} + \text{SE SS lbs/d}}$$

$$= \frac{(2222 \text{ mg/L})(\overbrace{0.460 + 0.178 \text{ MG}}^{0.638})(8.34)}{1610 + 240 \text{ lbs/d}}$$

$$= \frac{11823.08424 \text{ lbs}}{1850 \text{ lbs/d}} = \boxed{6.4 \text{ days}}$$

18. Determine MCRT given the following information:

Aeration Basin = 350,000 gal
Final Clarifier = 125,000 gal
Flow = 1,400,000 gpd
WAS Pump Rate = 27,000 gpd

MLSS = 2910 mg/L
S.E. SS = 16 mg/L
WAS = 6210 mg/L

★ Use 4th MCRT formula★

$$\text{MCRT} = \frac{(2910\text{ mg/L})\overbrace{(0.35+0.125\text{ MG})}^{0.475}(8.34)}{(6210\text{ mg/L})(0.027\text{ MGD})(8.34)+(16\text{ mg/L})(1.4\text{ MGD})(8.34)}$$

$$= \frac{11527.965\text{ lbs}}{1398.3678+186.816} = \frac{11527.965}{1585.1838} = \boxed{7.3\text{ d}}$$

Wasting Rates

19. Using Constant F/M Ratio: The desired F/M ratio for an activated sludge system is 0.5 lbs BOD/lb MLVSS. It has been calculated that 3400 lbs of BOD enter the aeration basin daily. If the volatile solids content of the MLSS is 69%, how many lbs MLSS are desired in the aeration basin?

$$\text{Desired MLVSS, lbs} = \frac{3400\text{ lbs BOD}}{0.5} = 6800\text{ lbs}$$

$$\text{Desired MLSS, lbs} = \frac{6800\text{ lbs}}{0.69} = \boxed{9855\text{ lbs}}$$

20. Using Constant MCRT: The desired MCRT for an activated sludge plant is 9 days. The secondary effluent flow is 3,220,000 gpd with a suspended solids content of 23 mg/L. There is a total of 32,400 lbs SS in the system. How many lbs/day WAS SS must be wasted to maintain the desired MCRT?

$$\text{WAS, lbs/d} = \frac{32{,}400\text{ lbs}}{9\text{ days}} - (23\text{ mg/L})(3.22\text{ MGD})(8.34)$$

$$= 3600\text{ lbs/d} - 617.6604\text{ lbs/d}$$

$$= \boxed{2982\text{ lbs/d}}$$

21. Given the following data, determine the lbs/day suspended solids to be wasted:

Aeration Tank Volume = 1.2 MG
Influent Flow = 3,100,000 gpd
BOD = 110 mg/L

Desired F/M = 0.4
MLSS = 2200 mg/L
%VS = 68%

$$\text{Desired MLVSS, lbs/d} = \frac{(110\ \text{mg/L})(3.1\ \text{MGD})(8.34)}{0.4}$$

$$= 7109.85\ \text{lbs}$$

$$\text{Desired MLSS,} = \frac{7109.85}{0.68} = 10455.66176\ \text{lbs}$$

$$\text{Actual MLSS} = (2200\text{mg/L})(1.2\ \text{MG})(8.34) = 22017.6\ \text{lbs}$$

$$\text{MLSS to Waste} = 22017.6 - 10455.6 = \boxed{\text{11,562 lbs MLSS to waste}}$$

Answers:

1. 1761 lbs BOD/day
2. 5726 lbs COD/day
3. 4459 lbs BOD/day
4. 6105 lbs COD/day
5. 8095 lbs MLSS
6. 10,870 lbs MLVSS
7. 7698 lbs MLVSS
8. 9025 lbs MLSS
9. 9899 lbs MLVSS
10. 0.43
11. 0.40
12. 0.16
13. 7116 lbs MLVSS
14. 7379 lbs MLVSS
15. 8.8 days
16. 8.5 days
17. 6.4 days
18. 7.3 days
19. 9855 lbs MLSS desired
20. 2982 lbs MLSS to waste
21. ~~3977~~ lbs MLSS to waste
    11,562