



KAFR EL SHEIKH WATER AND SANITATION COMPANY

WASTE WATER TREATMENT TRAINING PROGRAM

WASTEWATER TREATMENT LABORATORY TRAINING

2009

SAMPLING AND SAMPLE PRESERVATION

- The value of sampling
- The purpose of sampling
- Preparation of a Sampling Plan

A sampling plan should address the following questions:

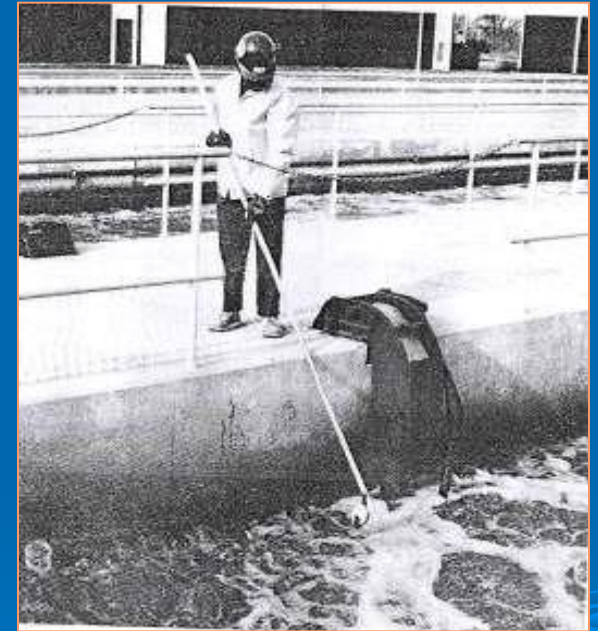
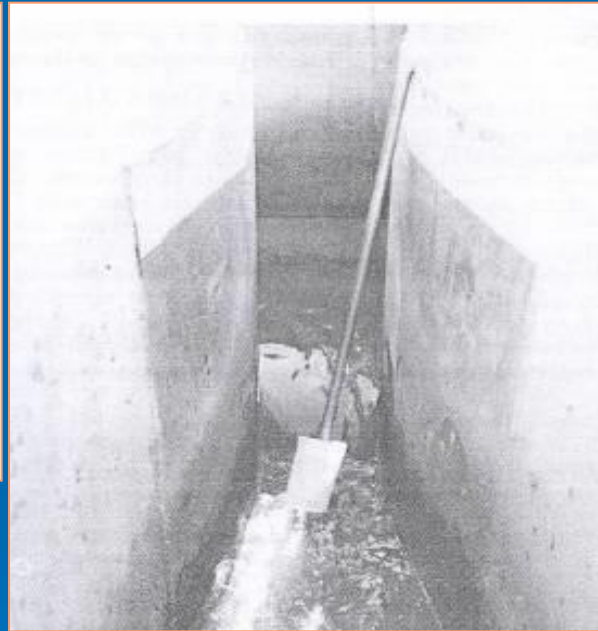
1. Why?
2. What?
3. Where?
4. How?
5. How frequently?
5. When?

Types of sample collection

1- Grab Sample



Samples should not be taken in areas of floating scum or debris



The downstream area of a Parshall flume or in the aeration basin are well mixed and thus good locations for sampling.

2- Composite Sample

➤ Unweighted composite sample.

Time	Flow, gpd	Volume in bottle
8:00 am	40,000	880 ml
9:00 am	80,000	880 ml
10:00 am	100,000	880 ml
11:00 am	200,000	880 ml
12:00 pm	120,000	880 ml
1:00 pm	60,000	880 ml



FLOW PROPORTIONAL SAMPLE (Weighted Composite Sample)

➤ Weighted composite sample

General composite procedure:

- 1. Decide the sample volume that will be needed for analysis.
- 2. Collect the samples.
- 3. Record the flow reading for each time a sample is taken.

The formula is:
$$\frac{\text{Flow at time} \times \text{volume of sample needed}}{\text{Total flow during composite period}}$$

FLOW PROPORTIONAL SAMPLE (WEIGHTED SAMPLE)

- **For example**, determine how much sample should be composited from each bottle. Assume 2000 ml should be collected for the analysis and the total flow is 600,000 m³.

Using the formula, the amount of sample that should be removed from sample container at 8:00am is:

$$\frac{40,000 \text{ m}^3 \times 2000 \text{ ml}}{600,000 \text{ m}^3} = 133 \text{ ml}$$

Time	Flow, gpd	Volume in bottle	Volume to composite
8:00 am	40,000	880 ml	133 ml
9:00 am	80,000	880 ml	267 ml
10:00 am	100,000	880 ml	333 ml
11:00 am	200,000	880 ml	667 ml
12:00 pm	120,000	880 ml	400 ml
1:00 pm	60,000	880 ml	200 ml

When these volumes are added to the sample container, the final volume will be 2000 ml.

BASICS OF SAMPLE COLLECTION AND ANALYSIS PLAN

1. Safety First.
2. Prepare the sample bottles
3. Site Selection
4. Sample Identification
5. Sample Collection
6. Sample Analysis
7. Preservation, Storage, and Holding Times
8. Laboratory Records and Reports

Sample Handling and Preservation				
Determination	Container	Min. Sample Size (ml)	Preservation	Max. Storage/ Recommended
Total Alkalinity	P, G	200	Refrigerate	24 h/14 d
BOD ₅	P, G	1,000	Refrigerate	6 h/48 h
Bacteriological	Sterilized Bottle	100	Refrigerate	6 h/6 h
O ₂ Dissolved				
Electrode	G, BOD Bottle	300	Analyze in situ	0.5 h
Winkler			Titration may be delayed after acidification	8 h/8 h
COD	P, G	100	Add H ₂ SO ₄ to pH <2 -refrigerate	7 d
Sulfides	P, G	100	add 4 drops 2N zinc acetate/100ml and NaOH to pH 9	7 d
Ammonia	P, G	500	Add H ₂ SO ₄ to pH <2 -refrigerate	7 d
pH	P, G	-----	Analyze immediately	2 h
O&G	G	500	Acidify and refrigerate	7d
TS, TSS, VSS, TDS	P, G	200	Refrigerate	7 h
Temperature	P, G	-----	Analyze in situ	NO
Sludge Metals	P, G	200	Refrigerate	24 h/14 d

WASTEWATER TREATMENT PLANT

DATE: ____/____/____

SAMPLE CUSTODY SHEET				
SAMPLE ID	TIME	SAMPLED BY	METHOD OF PRESERVATION	
			REFRIGERATOR/ICE CHEST ¹	ADD H ₂ SO ₄ To pH < 2
			☐	☐
			☐	☐
			☐	☐
			☐	☐
			☐	☐
			☐	☐
			☐	☐
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			☐	☐
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			☐	☐
			☐	☐
			☐	☐
Delivered to laboratory by:		Date:	Time:	
		Signed		
Received at laboratory by:		Date:	Time:	
		Signed		
Remarks:				

* Ice chest is considered to be Dark Preservation

WASTEWATER TREATMENT PLANT
Influent/Effluent Daily/Monthly Report of Analysis for the Month of: _____

Date	Day of Week	INFLUENT									EFFLUENT								
		Flow (x1,000) m ³ /d	pH SU	Alkalinity mg/l	TDS mg/l	VSS mg/l	TSS mg/l	BOD ₅ mg/l	T.Coliforms N/100 ml	F.Coliforms N/100 ml	pH SU	Alkalinity mg/l	TDS mg/l	VSS mg/l	TSS mg/l	BOD ₅ mg/l	T.Coliforms N/100 ml	F.Coliforms N/100 ml	
1																			
2																			
3																			
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31																			
Minimum Daily:																			
Maximum Daily:																			
Monthly Average:																			
											Monthly Average Corrected for Algae:								

BOD₅ is the amount of dissolved oxygen consumed by microorganisms, over a 5-day period, while degrading the organic constituents in the wastewater.

WASTEWATER TREATMENT PLANT LABORATORY

TOTAL SUSPENDED SOLIDS (TSS & VSS) WORK SHEET

SAMPLE DATE: / /

ANALYSIS DATE: / /

20th Edition, *Standard Methods for the Examination of Water and Wastewater*

PROCEDURE NUMBER 2540 D & 2540 E

ANALYST:

(1) SAMPLE TYPE/ID

(2) CRUCIBLE / FILTER NUMBER

(3) SAMPLE VOLUME, mL

(4) WT. OF DRY SOLIDS / CRUCIBLE / FILTER, g

(5) TARE WT. OF ASH SOLIDS / CRUCIBLE / FILTER, g

(6) TARE WT. OF CRUCIBLE / FILTER, g

(7) WT. OF DRY SOLIDS, g = (4) - (6)

(8) WT. OF VOLATILE SOLIDS, g = (4) - (5)

(9) TSS, mg/L = [(7) X 1,000,000] / (3)

(10) VSS, mg/L = [(8) X 1,000,000] / (3)

(11) % VOLATILE = [(10) X 100] / (9)

Lab Chemist and Technician Responsibilities

Certified Chemists and Lab Technicians help Operators monitor the Waste Water Treatment Plant.

Lab Technician duties are:

- Collect and preserve samples
- Prepare samples for analysis
- Keep the lab clean.

Lab Chemist duties are:

- Analyze samples
- Operate and maintain laboratory equipments and instruments
- Handle chemicals and wastes
- Data keeping and Reporting
- Quality Assurance/Quality Control (QA/QC)
- Manage Laboratory Budget.
- Laboratory Safety
- Any other related tasks requested by the plant manager.

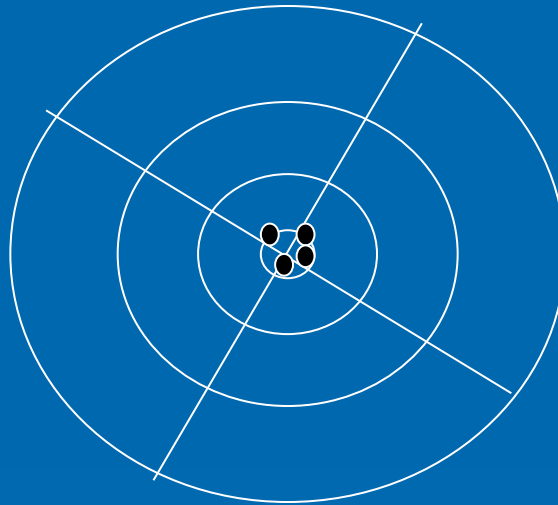
Laboratory Quality Assurance/Quality Control

Considerations regarding Quality of Data:

- Sampling Site
- Sampling Technique
- Sample Storage
- Transportation
- Analytical Method.
- Analytical Calculations.

Accuracy

- Accuracy measures how closely the analytical result, or the average set of analytical tests, approaches the actual value of the parameter being measured



Errors affecting the accuracy of the analytical measurements can be classified as:

- Systematic Errors
- Random Errors

Precision

Precision measures how closely a series of replicate measurements approaches the average. It is a measure of how well the results can be reproduced.

It is possible to have

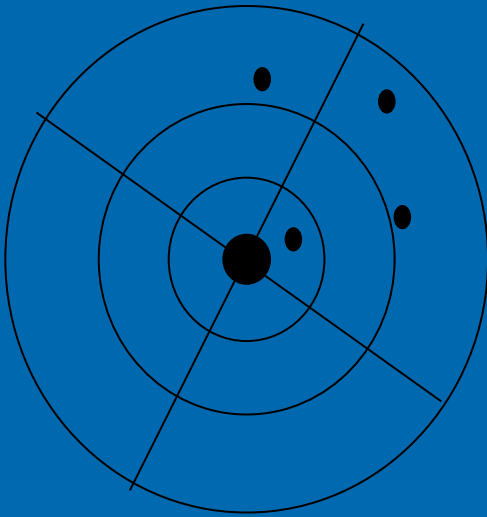
- excellent precision and poor accuracy.

Or conversely,

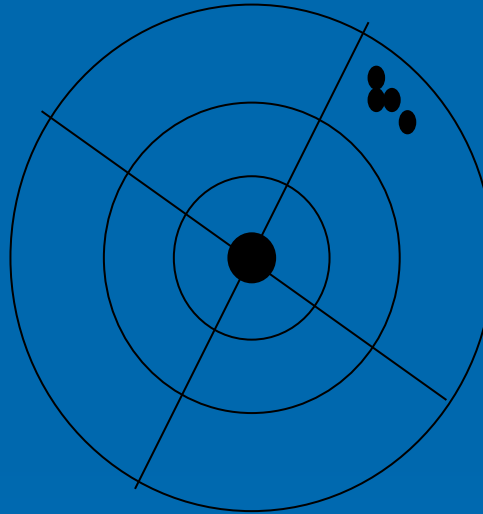
- excellent accuracy and poor precision.

Consequently, it is necessary to control both accuracy and precision to insure that reliable data is produced.

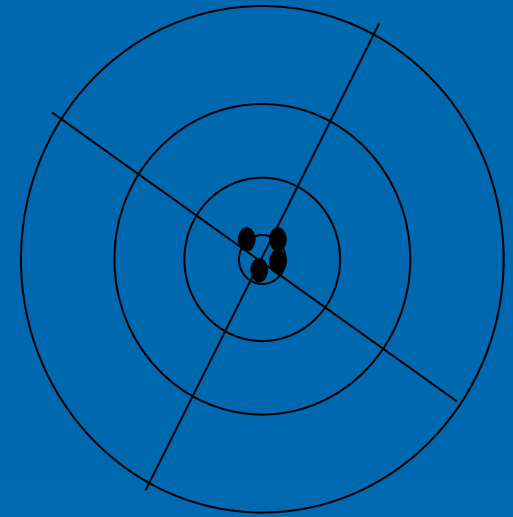
Accuracy and Precision



Low Accuracy, Low Precision
Low Bias



Low Accuracy, High Precision
High Bias



High Accuracy, High Precision
No Bias

General Laboratory Quality Control Procedures

Factors affecting Accuracy of Analytical Results:

- Preventive Maintenance of Lab Equipment
- Analytical Method used
- Preparation of Standard Solutions
- Standardization of Titrants.
- Preparation of Standard Curves.

General Laboratory Quality Control Procedures

Enter into Standard Solutions Logbook

- Date of preparation
- Name of person who prepared the standard
- Preparation procedures
- Source of chemicals used
- Expiration date
- Storage requirements

General Laboratory Quality Control Procedures

Standard solutions labeling

- Solution strength
- Date of preparation
- Expiration date
- Name of person who prepared the standard

*Chemicals must be stored in tightly closed containers and in a cool dry location.
All chemical solutions must be labeled clearly and the date the container was opened should be recorded on the label.*

General Laboratory Quality Control Procedures

- Chemicals should be monitored using the Chemical Control Log.
- Chemical Inventory should be monitored using the Chemical Inventory Log
- The appropriate quality of water must be used for preparing reagents, dilutions and for cleaning glassware.

Examples of QA/QC sheets

WASTEWATER TREATMENT LABORATORY

Equipment Calibration Sheet

Equipment: _____

Identification No: _____

Calibration Method: _____

Calibration Frequency: _____

Readings			Calibrated By		
Desired	Actual	Percent Difference	Signature	Date	Remarks

WASTEWATER TREATMENT LABORATORY

Thermometer Calibration Log

Date	Thermometer ID #	Reading at Boiling ° C	Reading in Ice Bath ° C	Reading of NBS Certified Thermometer ° C	By

WASTEWATER TREATMENT LABORATORY Distilled Water Suitability Log

Parameter				Checked By		
pH	Conductivity μS/cm	Total Solids mg/L	Bacteria Count N/100 mL	Signature	Date	Time

WASTEWATER TREATMENT LABORATORY

Chemical Control Log

Item	Qty.	Unit	Expiration Date	Date Opened	Remarks

Required Quality Control Analysis				
Parameter	Blank	Standard	Duplicate	Spike
PH*		X	X	
BOD	X	X	X	X
DO*			X	
TS	X	X	X	
TSS	X		X	
TDS	X	X	X	X
COD	X	X	X	X
Alkalinity		X	X	
Coliforms	X		X	

Wastewater Treatment Laboratory
Duplicate Sample Analysis

Parameter: _____

Method: _____

[illegible]

n = Number of Pairs

Warning Control Limits = $\pm 2 S_d$ Action Control Limits = ± 3 Sd

$$\text{Standard Deviation (Std)} = \sqrt{\frac{(B - A)/n}{1 - 1}}$$

Parameters and Tests used in Waste Water Treatment Operations

- 1- Temperature
- 2- pH
- 3- Color
- 4- Alkalinity
- 5- T. Chlorine
- 6- DO
- 7- TS&TVS
- 8- TSS&VSS
- 9- Settleometer test
- 10- Settleable solids
- 11- O&G
- 12- Sulfides
- 13- Nitrates
- 14- Centrifuge test
- 15- Microscopic Examination
- 16- Ammonia nitrogen
- 17- BOD₅
- 18- COD
- 19- T. Coliforms

1- TEMPERATURE

TEMPERATURE SCALES

- The Celsius scale is used for most temperature measuring purposes.
- The Fahrenheit scale is usually used in the United States .
- The Kelvin scale, or absolute zero is usually used for scientific measurements.

Temperature Conversion Table

From	To Fahrenheit	To Celsius	To Kelvin
Fahrenheit (F)	F	$(F - 32) * 5/9$	$(F - 32) * 5/9 + 273.15$
Celsius (C or °)	$(C * 9/5) + 32$	C	$C + 273.15$
Kelvin (K)	$(K - 273.15) * 9/5 + 32$	$K - 273.15$	K

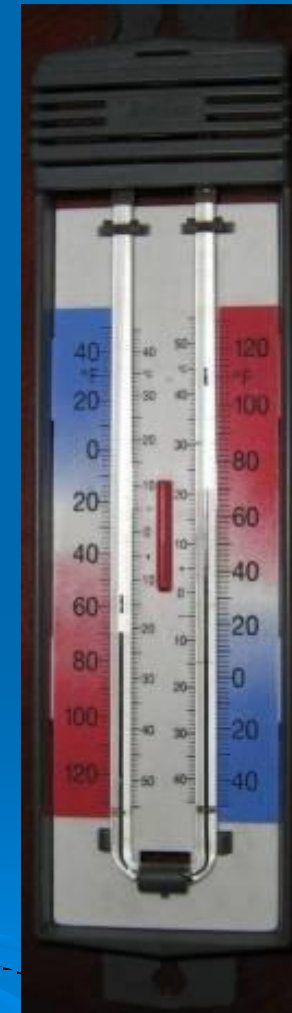
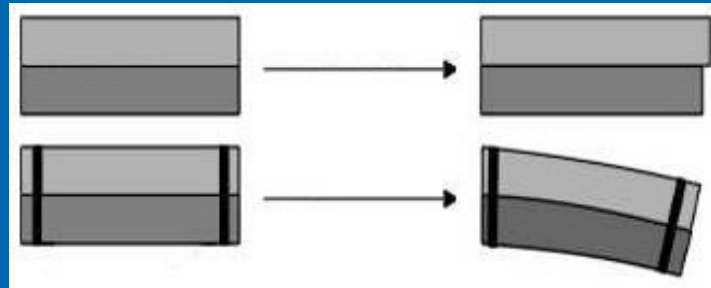
Thermometers

A thermometer is essential to control the temperature during many experiments such as BOD5, COD, bacteriological tests and many others tests where temperature can accelerate or slow down the reaction.

Common types of thermometers:

- Alcohol thermometer
- Bi-metal mechanical thermometer
- Infrared thermometer
- Liquid crystal thermometer
- Medical thermometer (e.g. oral thermometer)
- Mercury-in-glass thermometer
- Resistance thermometer
- Six's thermometer- also known as a **Maximum minimum thermometer**
- Thermocouple

EXAMPLES OF SOME THERMOMETERS



Calibration

- Periodically check the thermometer against a precision thermometer certified by the National Institute of Standards and Technologies (NIST)
- In case of inaccurate readings, a correction chart can be created by using the NIST thermometer.

Temperature Measurement

refer to the Procedures Manual Method No.1

2- pH Measurement

DEFINITION OF PH

pH is a measure of the acidity or basicity of a sample. Pure water dissociates into hydrogen ions and hydroxide ions.



$$(\text{H}^+) = 10^{-\text{pH}}$$

$$\text{pH} = -\text{Log}_{10} (\text{H}^+)$$

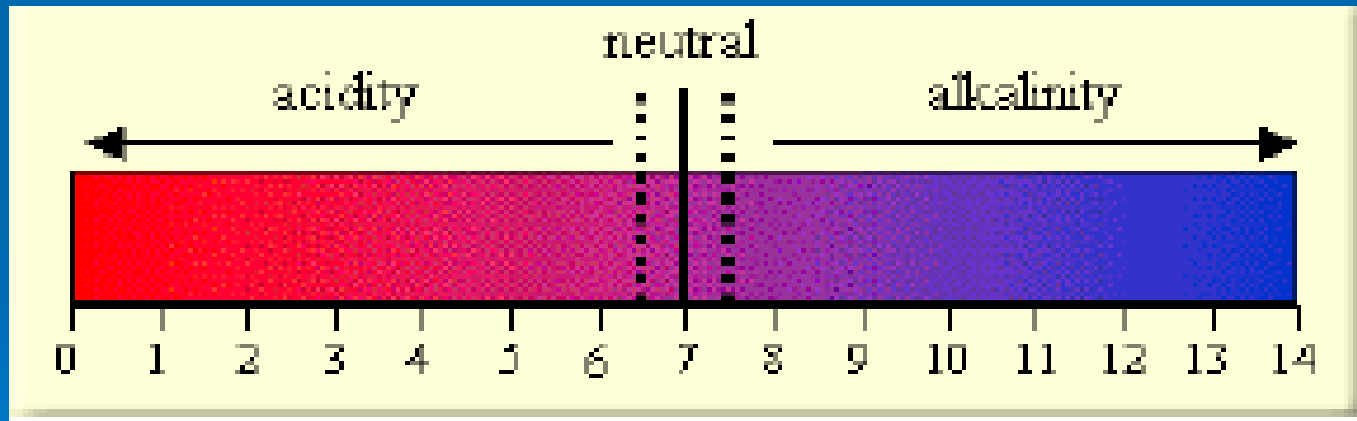
A scale of 0 to 14 is used for measurement, with 0 being extremely acidic and 14 being extremely alkaline. The midpoint (7.0) is neutral.

i.e.

pH = 0 highly acidic

pH = 7 neutral

pH = 14 highly basic



- Importance of pH measurements

- Sample Handling

SAFETY: Use latex gloves when collecting and handling all wastewater samples

- Equipment

pH meter



Electrode Calibration

Electrode Storage

pH Measurement

Refer to the Procedures Manual Method No.2-1

Precautions

- pH should be measured as soon as possible after sample collection, preferably within 15 minutes
- If sample must be stored, it should be refrigerated at 4 ° C with no preservatives added and the test must be performed no later than 6 hrs after collection
- If pH values vary widely, standardize before measurement with a buffer solution having a pH within 1 to 2 pH units of the sample

3- COLOR

- Scope and application

- Interferences

- Sample handling

- Summary of Method

- Procedures

Refer to the Procedures Manual Method No. 3

Sampling

- Samples must be collected in plastic or glass bottles. Analyze the sample as soon as possible; fill the sample bottle completely. Avoid excessive agitation or prolonged contact with air.

Sample Preservation

- Samples can be stored for 24 at 4°C but must be warmed up to room temperature before measurements.

Accuracy check

- Making use of standard solution (500 units) a series of dilutions can be used to achieve more accurate results.

4- ALKALINITY

- SCOPE AND APPLICATION
- INTERFERENCES
- SAMPLE HANDLING

Summary of Method

Two titration procedures exist for the determination of alkalinity:

- (1) Potentiometer titration using a pH meter and titrating to specific endpoints, or
- (2) Titration using traditional pH indicators.

Refer to the Procedures Manual Method No.4

REPORTING

- Report the alkalinity of raw water and treated water once per day on the Daily Chemical Analysis Report.
- Results should be reported to the nearest one unit
(for example, 146 mg/L as CaCO_3).

QUALITY CONTROL

Results can be controlled by analyzing a duplicate sample.

5-TOTAL RESIDUAL CHLORINE

- SCOPE AND APPLICATION

- DEFINITIONS

What is Total Chlorine?

What is Free Available Chlorine (FAC)?

What is Combined Chlorine?

What is Total Residual Chlorine (TRC)?

What is Free Available Residual Chlorine (FRC)

What is Combined Residual Chlorine?

- What is Chlorine Demand?
- What is Chlorine Requirement?
- What are Chloramines?

4500 CL - IODOMETRIC METHOD

Principle of Method

Interferences

Minimum detectable concentration

The minimum detectable concentration is approximately 40 µg Cl as Cl₂/L if 0.01 N Na₂S₂O₃ is used with a 1000-mL sample.

Refer to the Procedures Manual Method No.5

6-DISSOLVED OXYGEN (DO)

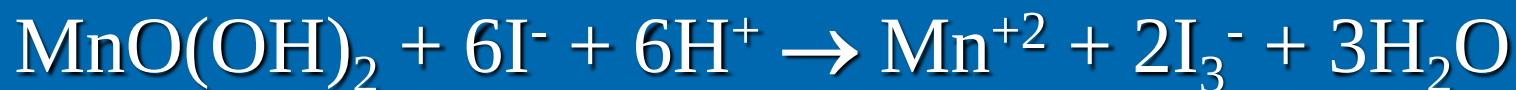
- Environmental Significance of DO.
- Dissolved Oxygen Measurement

Two methods for DO analysis exist :

- 1-The Winkler or iodometric method and its modifications
- 2-The electrometric method using membrane electrodes.

1-Winkler or iodometric method

REACTIONS:



Winkler or iodometric method

Reagents

1-MANGANOUS SULFATE REAGENT

2- ALKALI-IODIDE-AZIDE REAGENT.

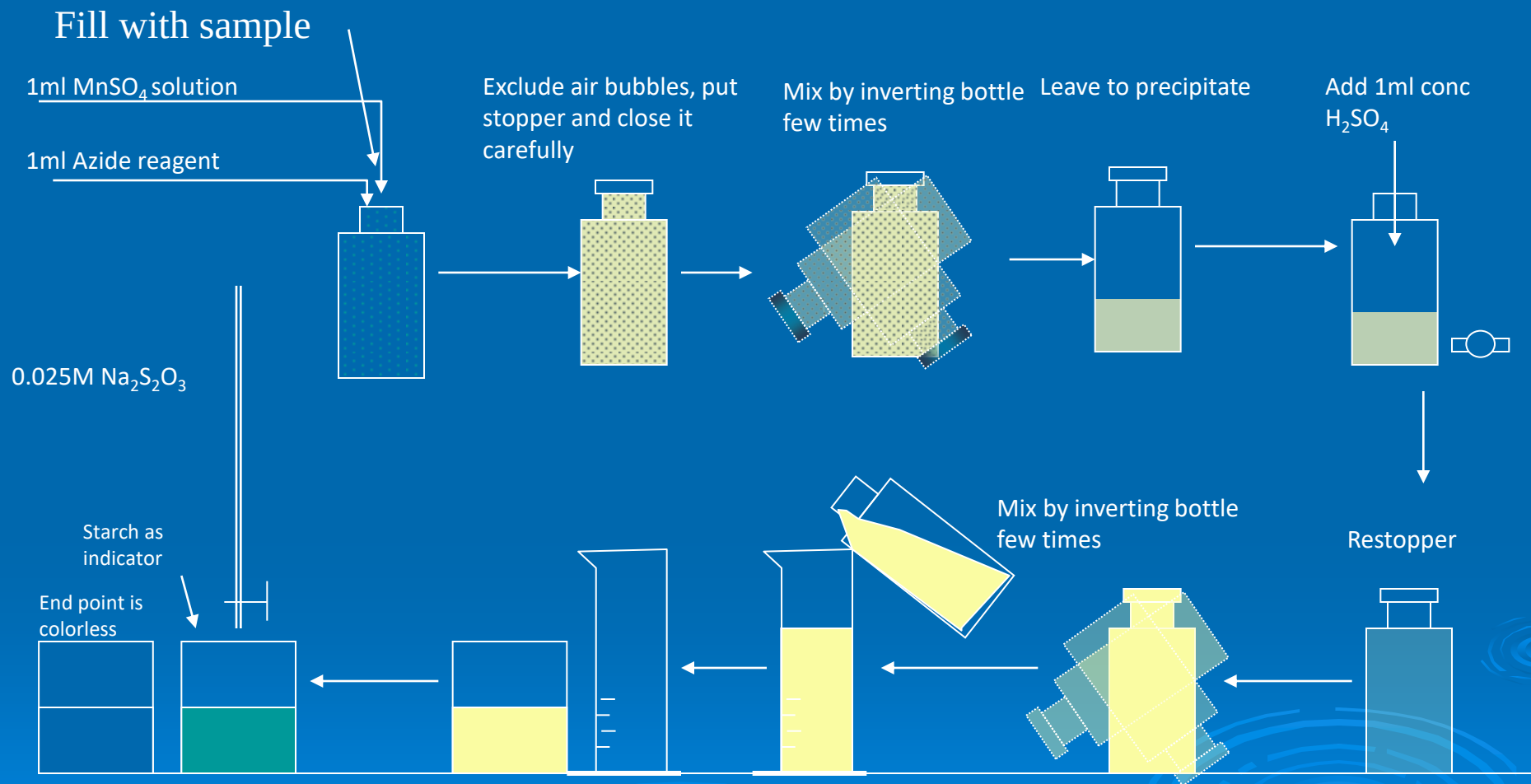
3- SULFURIC ACID CONC.

4- STARCH INDICATOR

5- STANDARD SODIUM THIOSULFATE TITRANT.

*For analytical procedure refer to the Procedures Manual
Method No.6-1*

Winkler or iodometric method



2- The electrometric method using membrane electrodes.

- It is better to measure the DO in situ using a portable DO meter.



DO Meter Calibration

- 1- Correlation between several samples
- 2- Calibration in moist air (100% humidity)
- 3- Calibration in air saturated water

DO Measurement

➤ Sample collection.

➤ Using the probe.

For DO measurements refer to the Procedures Manual Method No.6-2

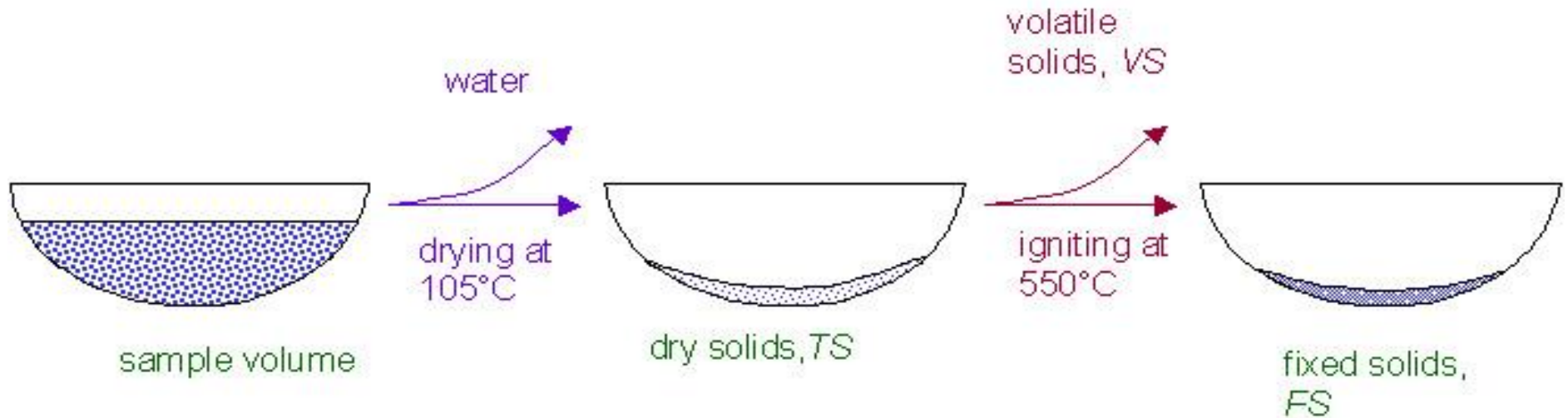
7- TOTAL SOLIDS AND TOTAL VOLATILE SOLIDS (TS AND TVS)

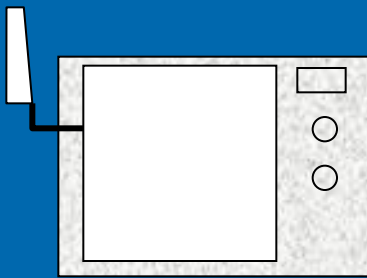
DEFINITION

- **“Total solids”**: the residue left in the vessel after evaporation of liquid from a sample and subsequent drying in an oven at 103 °C to 105 °C.
- **“Volatile solids”**: The weight loss after a sample is ignited (heated to dryness at 550 °C).

For methods refer to the Procedures Manual Method No.7

General Schematic of steps to determine *TS*, *TVS*, and *TFS*.





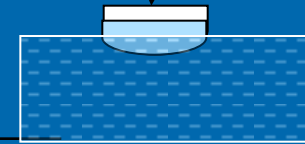
1. Ignite empty dish in muffle furnace



2. Cool in desiccator



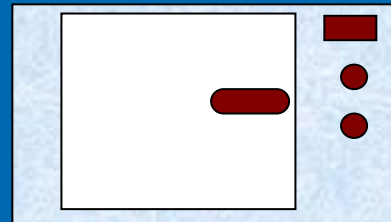
3. Weigh empty dish



4. Place a volume of sample in a prepared evaporating dish. Then evaporate on a water bath



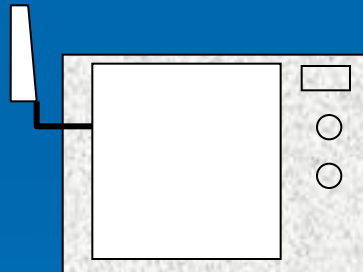
6. Cool in desiccator



5. Dry the residue in oven at 103°C to 105°C for 12 hours.



7. Weigh dish and dry residue



8. Ignite empty dish in muffle furnace



9. Cool in desiccators



10. Weigh dish and ash.

Outline Of Procedure For Total And Volatile Solids

Balance Calibration and Standardization

- Calibrate the analytical balance at 2 mg and 1000 mg using class "S" weights.
- Calibration shall be within $\pm 10\%$ (i.e. ± 0.2 mg) at 2 mg and $\pm 0.5\%$ (i.e. ± 5 mg) at 1000 mg.
- If values are not within these limits, recalibrate the balance.
- Place a 50 g weight and a 2 mg on the balance. Verify that the balance reads not more than $50.002 \pm 10\%$ (i.e. ± 0.2 mg).

Calculations

Calculate the grams of Total and Volatile solids as follow:

Sample volume ml = X

Wt of the Empty Dish g = W1

Wt of dish + Residues g = W2

Wt of dish + Ash g = W3

$$\text{TS g/l} = (W2 - W1) \times \frac{1000}{X \text{ ml}}$$

$$\text{TVS g/l} = (W2 - W3) \times \frac{1000}{X \text{ ml}}$$

Calculation Example

- Calculate the Total and Volatile solids as follow:
- Sample volume = 200 ml
- Wt of the Empty Dish = 50.3552 g
- Wt of dish + Residues = 50.3794 g
- Wt of dish + Ash = 50.3685 g
- TS g/l
$$= (50.3794 - 50.3552) \times \frac{1000}{200} = 0.121g / l = 121mg / l$$
- TVS g/l
$$= (50.3794 - 50.3685) \times \frac{1000}{200} = 0.0545g / l = 54.5mg / l$$

PRECAUTIONS

- Make and keep samples homogeneous during transfer.
- The temperature at which the residue is dried has an important bearing on results
- Organic matter may be lost by volatilization, but not completely destroyed.
- Loss of CO_2 results from conversion of bicarbonates to carbonates and carbonates may be decomposed partially to oxides or basic salts.
- Some chloride and nitrate salts may be lost.

TOTAL SOLIDS & VOLATILE SOLIDS (TS & TVS) WORK SHEET				SAMPLE TYPE : WASTEWATER				
20 th Edition, <i>Standard Methods for the Examination of Water and Wastewater</i>				ANALYST: <i>MAGDY MOKHTAR</i>				
PROCEDURE NUMBER 2540 D & 2540 E								
(1) SAMPLE TYPE/ID	1	2	3	4				
(2) CRUCIBLE NUMBER	12	7	9	10				
(3) SAMPLE VOLUME ml	100	100	100	100				
(4) WT. OF CRUCIBLE g	70.2555	1.0085	1.0087	1.0072				
(5) WT. OF DRY SOLIDS / CRUCIBLE , g	70.9115	2.4709	1.9196	2.3582				
(6) WT. OF ASH / CRUCIBLE g	70.4413	1.4454	1.4454	1.4138				
(7) WT. OF DRY SOLIDS, g = (5) - (4)	0.656	1.4624	0.9109	1.351				
(8) TS, mg/l = [(8) X 1,000,000] / (3)	6560	14624	9109	13510				
(9) WT. OF VOLATILE SOLIDS, g = (5) - (6)	0.1858	0.4369	0.4367	0.4066				
(10) TVS, mg/l = [(7) X 1000,000] / (3)	1858	4369	4367	4066				
% TVS / TS	28.3	29.9	47.9	30.1				

8- TOTAL SUSPENDED SOLIDS (TSS) AND VOLATILE SUSPENDED SOLIDS (VSS)

- A well mixed sample is filtered through a pre-washed, pre-weighed, glass fiber filter.
- The suspended solids are retained on the filter.
- The filter is then rinsed, dried, and weighed.
- The increase in weight represents the suspended solids.

Sample Collection

- TSS samples can be either composite or grab samples and can be collected in either glass or plastic bottles.
- Samples should be analyzed as soon as possible or preserved on ice or refrigerated to reduce microorganism activity.
- Holding time should not be longer than 7 days.



Total Suspended Solids and Volatile Suspended Solids

Using the Gooch Filtering Crucible Method

Sample can be from:

- Influent
- Secondary Effluent
- Activated Sludge Process:
Mixed Liquor or
Return or Waste Sludge

Common Ranges ,mg/l

150 (weak) - 400+ (strong)

10 (very good) - 60+ (bad)

Depending on Type of process

1000 - < 5,000

2000 - < 12,000

Equipment

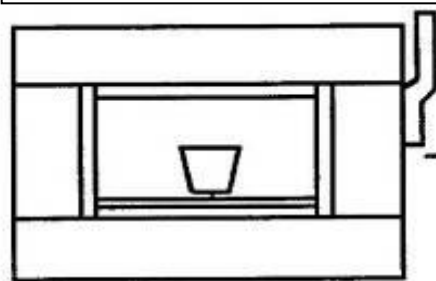
- 2.4 cm glass fiber filter. Whatman 934 AH, Whatman GF/A
- No.4 Gooch crucible.
- Distilled water.
- Filtration flask.
- Graduated cylinder.
- Vacuum pump or aspirator.
- Convection oven (TSS)
- Muffle furnace (VSS)
- Analytical balance

TSS samples should always be run in duplicate. It takes very little time to run a second sample.

Refer to the Procedures Manual Method No. 8 for details

Note : Larger particles determined by the lab technician to be "unrepresentative" must be removed prior to analysis. They cannot be removed from the filter after filtration.

OUTLINE OF PROCEDURE FOR SUSPENDED SOLIDS



1. Ignite dish and glass fiber filter at 550°C for 1hr in muffle furnace

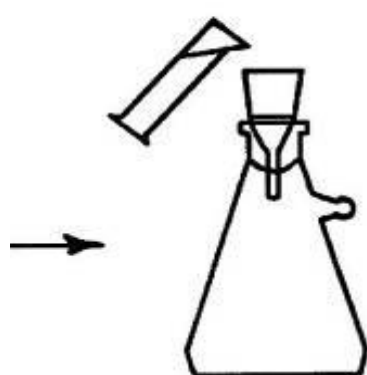


2. Cool.

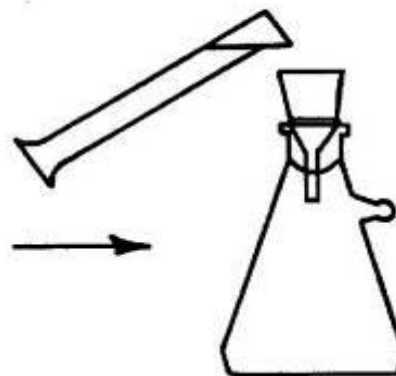


3. Weigh and store in desiccator.

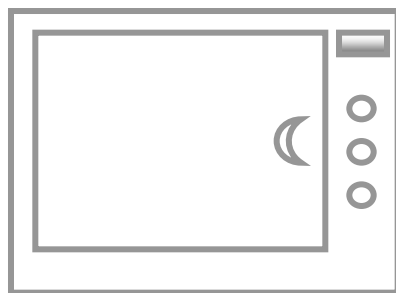
4. Put the crucible and the filter paper in the filtration funnel



5- Wash filter-crucible with distilled water.



6- Pour known volume of sample into crucible



7- Dry crucible-filter for 2 hours at 103°C



8- Cool in desiccators.



Calculations

- Calculate the grams of Total Suspended Solids as follows:
- Sample volume X (ml)
- Wt of dish + filter W1 (g)
- Wt of dish + filter +Residues W2 (g)

$$\text{TSS (g/l)} = (W2 - W1) * 1000/X$$

Calculation Example

- Calculate the grams of Total Suspended solids as follow:
- Sample volume ml = 100
- Wt of dish + filter g = 15.2330
- Wt of dish + filter +Residues g = 15.2561

TSS

$$= (15.2561 - 15.2330) \times \frac{1000}{100} = 0.231g / l = 231mg / l$$

PRECAUTIONS

- Exclude large floating particles or submerged agglomerates of non-homogeneous materials from the sample.
- For samples high in dissolved solids thoroughly wash the filter to ensure removal of dissolved material.
- Prolonged filtration times as a result of filter clogging may produce high higher TSS values owing to increased colloidal materials being captured on the clogged filter. Some chloride and nitrate salts may be lost.
- Choose sample volume to yield between 2.5 and 200 mg dried residue. If volume filtered fails to meet minimum yield, increase sample volume up to 1 L. If complete filtration takes more than 10 min, increase filter diameter or decrease sample volume.

Volatile Suspended Solids (VSS)

- Volatile Suspended Solids are defined as the suspended solids that can be ignited at 550°C.
- Volatile suspended solids represent the amount of organics present in the sample.
- Volatile suspended solids in the mixed liquor of an activated sludge system represent approximately the biomass.
- If volatile suspended solids are to be run, the glass fiber filters must be pre-ignited in the muffle furnace at 550 °C to remove any organic contaminants on the filter.

VSS Analysis

For details refer to the Procedures Manual Method No. 8-2

Safety: Use long forceps to place and remove the TSS filter from the muffle furnace.

VSS Calculations

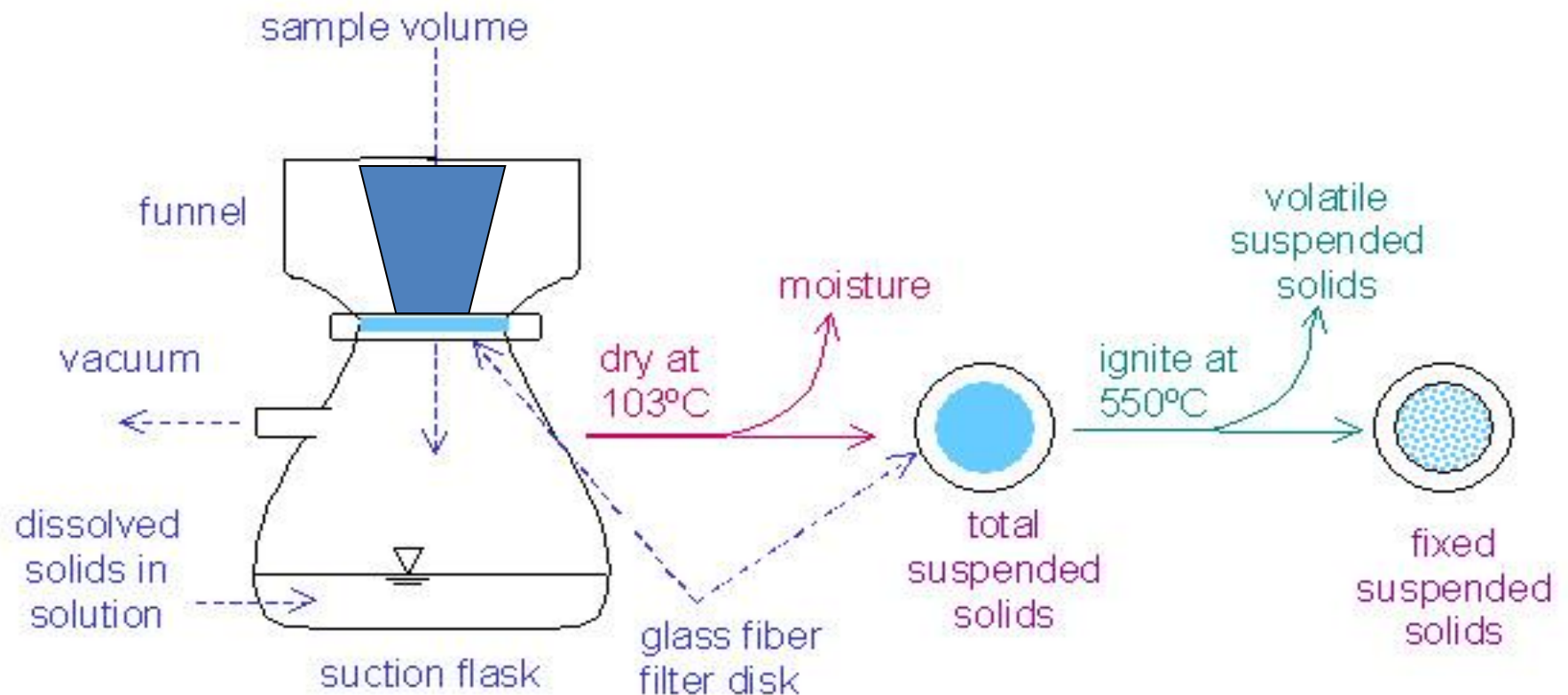
- Volume filtered = 45 ml
- Weight of TSS filter + dish = 1.4983 gm
- Weight of ash + dish (after ignition at 550°C) = 1.4956 gm
- Weight of VSS = 0.0027 gm

$$\text{VSS mg/L} = \frac{1.4983 - 1.4956}{45} \times 1000,000 = 60$$

WASTEWATER TREATMENT PLANT LABORATORY

TOTAL SUSPENDED SOLIDS (TSS & VSS) WORK SHEET				SAMPLE DATE: / /		ANALYSIS DATE: / /	
20th Edition, <i>Standard Methods for the Examination of Water and Wastewater</i> PROCEDURE NUMBER 2540 D & 2540 E				ANALYST:			
(1) SAMPLE TYPE/ID	INF	INF	EFF	EFF			
(2) CRUCIBLE / FILTER NUMBER	1	2	3	4			
(3) SAMPLE VOLUME, mL	10	5	500	200			
(4) WT. OF DRY SOLIDS / CRUCIBLE / FILTER, g	1.533 2	1.699 1	1.844 1	1.3362			
(5) TARE WT. OF ASH SOLIDS / CRUCIBLE / FILTER, g	1.529 1	1.697	1.843 5	1.336			
(6) TARE WT. OF CRUCIBLE / FILTER, g	1.525 2	1.695 3	1.84	1.3341			
(7) WT. OF DRY SOLIDS, g = (4) - (6)	0.008	0.003 8	0.004 1	0.0021			
(8) WT. OF VOLATILE SOLIDS, g = (4) - (5)	0.004 1	0.002 1	0.000 6	0.0002			
(9) TSS, mg/L = [(7) X 1,000,000] / (3)	800	760	8.2	10.5			
AVG TSS mg/l	780		9.35				
(10) VSS, mg/L = [(8) X 1,000,000] / (3)	410	420	1.2	1.0			
AVG VSS mg/l	415		1.1				
(11) % VOLATILE = [(10) x 100] / (9)	53.21%		12.83%				

General schematic of steps to determine *TSS*, *VSS*, and *FSS*.

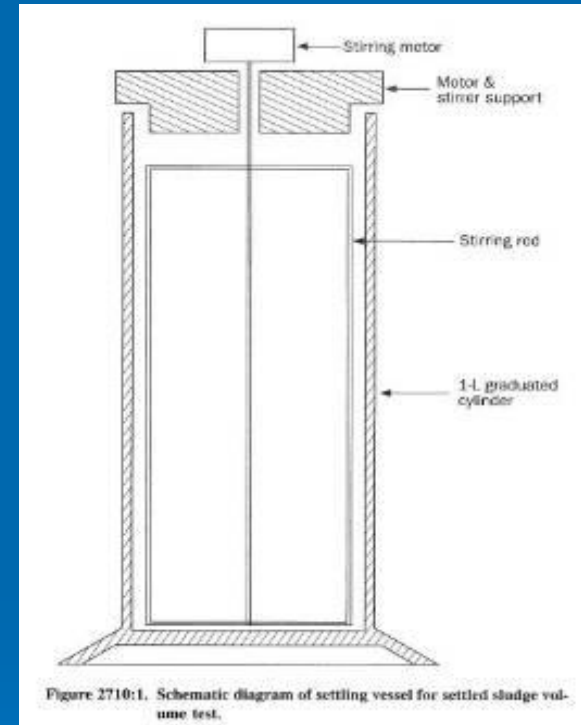


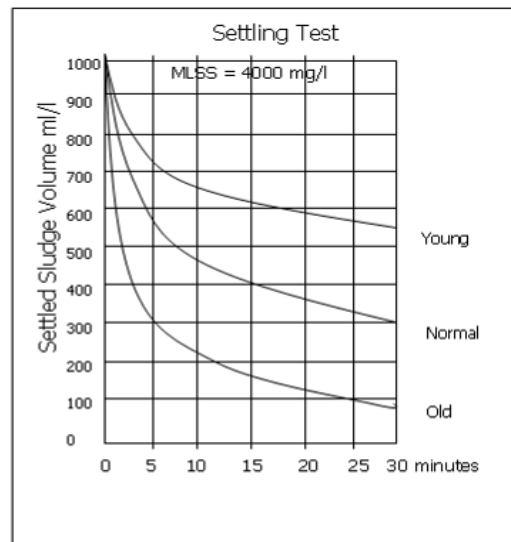
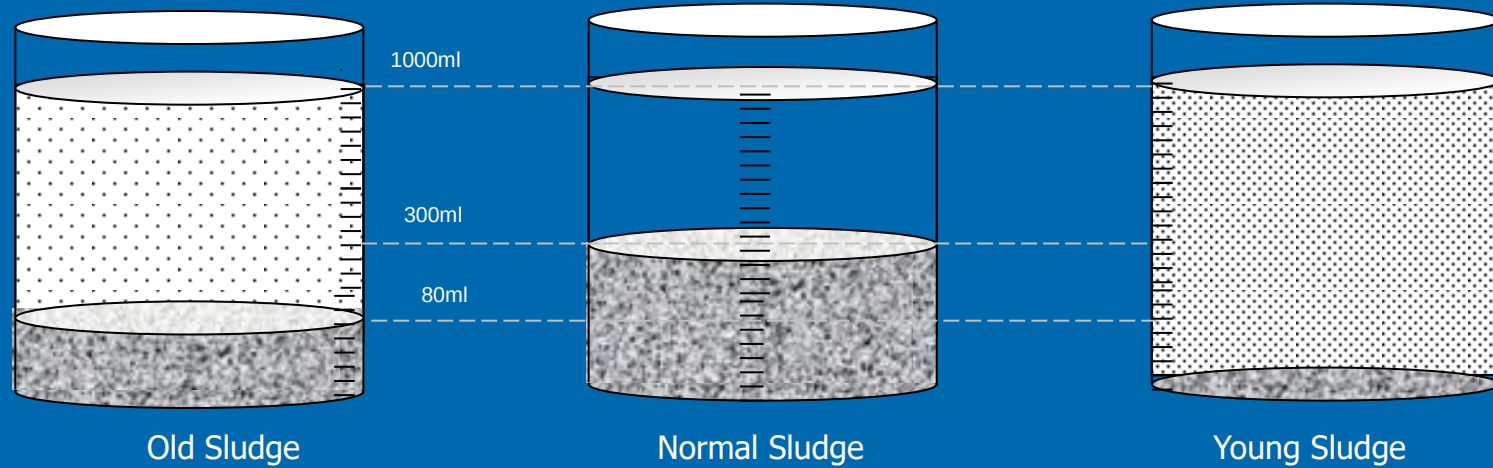
9- SETTLOMETER TEST

SCOPE AND APPLICATION

- Sludge settling characteristics
- Solids Inventory
- Waste Activated Sludge Volume

Refer to the Procedures Manual Method No. 9





Settleability Curve

CALCULATIONS

SSV_{30} is used in calculating the sludge volume index (SVI).

Where:

➤ For example:

- $SSV_{30} = 300 \text{ mL}$ and
- $MLSS = 2,500 \text{ mg/L}$

➤ Therefore: $SVI = (300/2,500) \times 1,000 = 120 \text{ mL/g}$

10- Settleable Solids

DEFINITION

Settleable Solids: Those solids in suspension which will pass through a 2000 micron sieve and settle in one hour under the influence of gravity.

Equipment:

Imhoff cone.

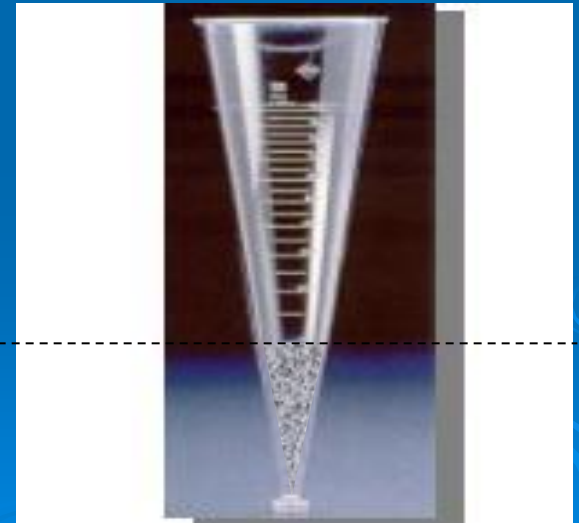


Test

Fill an Imhoff cone to the 1-L mark with a well-mixed sample. Settle for 45 min.

Gently agitate sample near the sides of the cone with a rod or by spinning, settle 15 min longer,

Record volume of settleable solids in the cone as milliliters per liter (ml/l). Make sure to look at the cone from the reading level.



11- OIL AND GREASE

DEFINITION

"Oil and grease" is defined as any material recovered as a substance soluble in **n-Hexan** as solvent. It includes other material extracted by the solvent from an acidified sample (such as sulfur compounds, certain organic dyes, and chlorophyll) and not volatilized during the test.

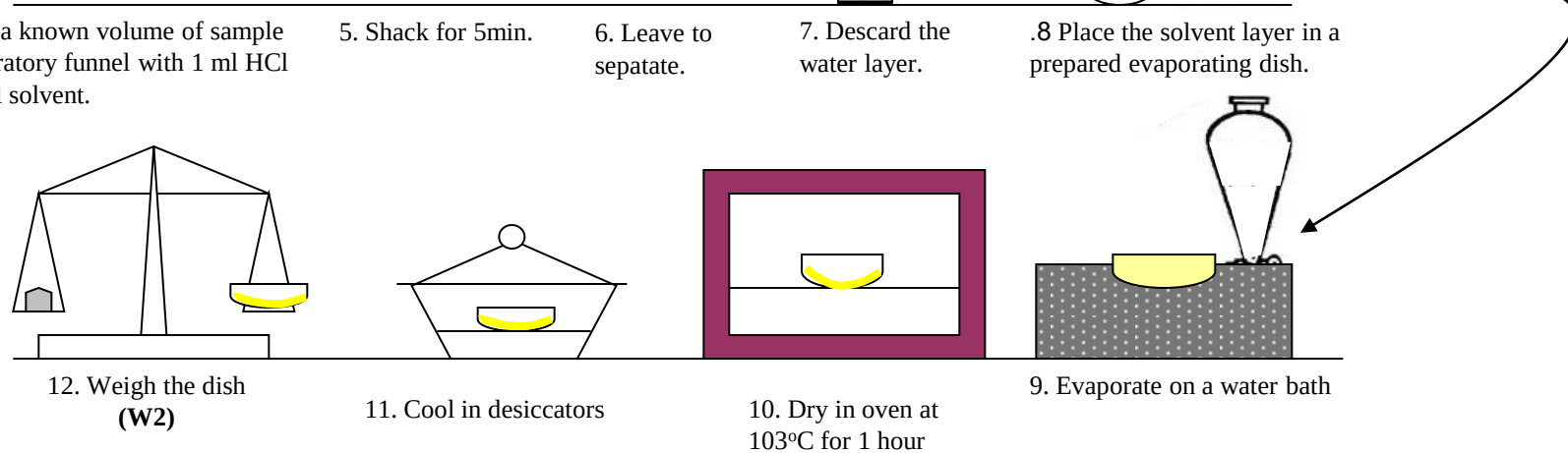
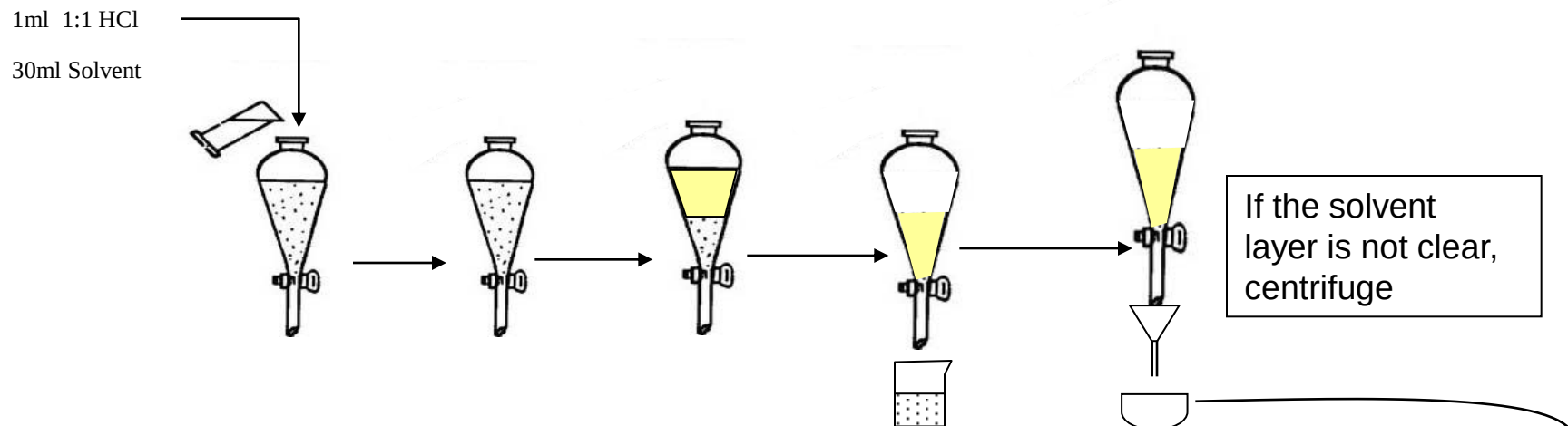
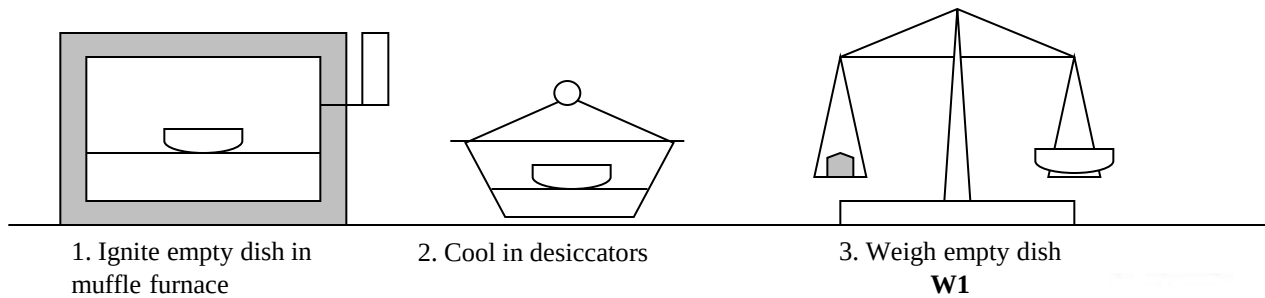
Sample Collection, Preservation, and Storage

- Collect a representative grab sample in a wide-mouth glass bottle that has been washed with soap, rinsed with water and rinsed with solvent to remove any residues that might interfere with the analysis.
- Do not overfill the sample container and
- Do not subdivide the sample in the laboratory.
- If analysis is to be delayed for more than 2 h, acidify to pH 2 (either 1: 1 HCl or 1: 1 H₂SO₄) and refrigerate.

EQUIPMENTS

- 1- Separatory funnel (2L) with TFE stopcock
- 2- Crucible (100 ml)
- 3- Funnel, glass
- 4- Filter paper 11 cm
- 5- Centrifuge 2400 rpm and centrifuge tubes
- 6- Water bath
- 7- Vacuum pump
- 8- Desiccator

For details refer to the Procedures Manual Method No. 11



Calculation

$$\text{mg/l Oil and Grease} = \frac{(W2 - W1) \times 1,000}{\text{ml sample}}$$

W2 (g) = Weight of dish with the dried residue

W1(g) = Tare weight of the empty dish

WASTEWATER TREATMENT PLANT

OIL AND GREASE WORK SHEET						SAMPLE DATE: / /				ANALYST:
<i>20th Edition, Standard Methods for the Examination of Water and Wastewater</i>										
PROCEDURE Number 2540 C						ANALYST:				
(1) SAMPLE TYPE	A1	A2	B1	B2						
(2) DISH NUMBER	1	2	3	4						
(3) SAMPLE VOLUME, mL	100	100	100	100						
(4) TARE WT. OF DISH,g	70.2335	71.568	70.9663	72.8521						
(5) WT. OF EXTRACTED O&G + DISH,g	70.2664	71.6022	71.124	72.9955						
(6) WT. OF EXTRACTED O&G,g = (5) - (4)	0.0329	0.0342	0.1577	0.1434						
(7) O&G, mg/L = [(6) X 1,000,000] / (3)	329	342	1577	1434						
(8) AVERAGE O&G mg/L	335.5		1505.5							

12- SULFIDES S^{2-}

Presence and Significance

Sampling and Storage

- Take sample with minimum aeration.
- Either analyze sample immediately after collection or preserve for later analysis with zinc acetate solution.

To preserve a sample for a total sulfide determination put zinc acetate and sodium hydroxide solutions into bottle before filling it with sample. Use 4 drops of 2N zinc acetate solution per 100 mL sample.

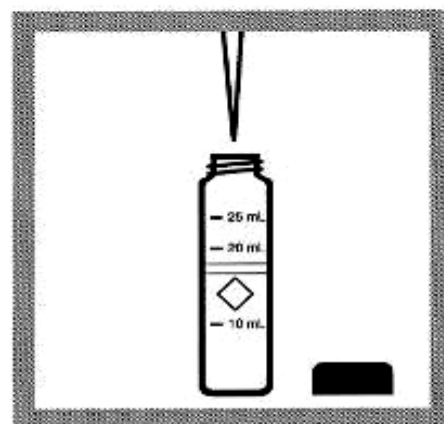
Hach Dr/2000 Spectrophotometer Method



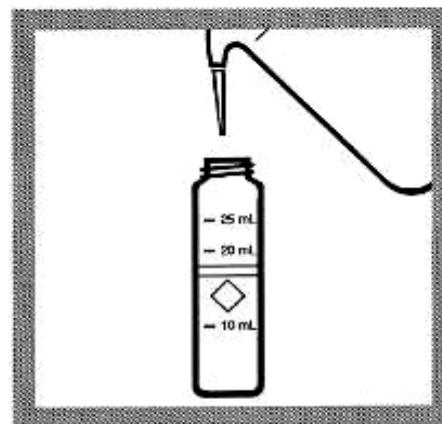
Method 8131

Hach Programs

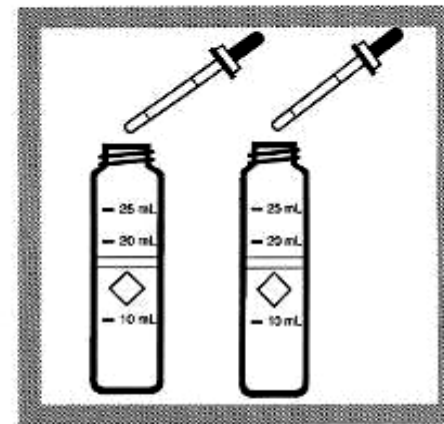
1. Touch
Hach Programs.
Select program
690 Sulfide.
Touch **OK.**



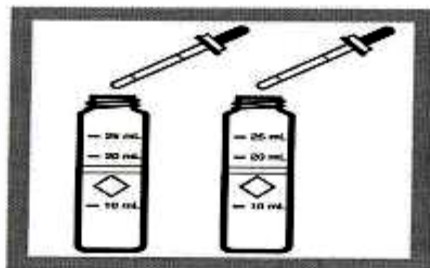
2. Avoiding excess agitation of the sample, use a pipet add 25 mL of sample to a sample cell (the prepared sample).



3. Measure 25 mL of deionized water into a second sample cell (the blank).

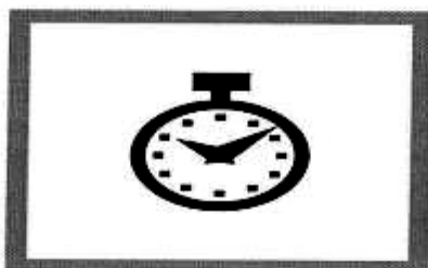


4. Use the calibrated 1-mL dropper to add 1.0 mL of Sulfide 1 Reagent to each cell. Swirl to mix.



5. Use the calibrated 1-mL dropper to add 1.0 mL of Sulfide 2 Reagent to each cell. Cap the cell and immediately invert to mix.

A pink color will develop, then the solution will turn blue if sulfide is present.



6. Touch the timer icon.
Touch **OK**.
A five-minute reaction period will begin.



7. When the timer beeps, wipe the blank and place it into the cell holder.



8. Touch **Zero**.
The display will show:
0 µg/L S²⁻



9. Wipe the prepared sample and place it into the cell holder.

Touch **Read**.

Results will appear in µg/L S²⁻.

13- NITRATES $\text{NO}_3\text{-N}$

- Nitrate is the highest oxidation state of nitrogen. It is being produced by nitrifying bacteria in waste water processes.

IMPORTANCE OF NITRATES IN THE ENVIRONMENT:

- The presence of nitrate in water indicates that the water may be polluted with organic wastes.
- Nitrates in the effluent serves as a fertilizer together with phosphates and can cause eutrophication in the receiving water (stimulate the growth of algae and water weeds)

SAMPLE HANDLING

- Samples should be collected in glass or plastic bottles with a volume of at least 100 mL.
- Samples should be analyzed as soon as possible after collection (within 1 hour), but may be stored at 4°C for up to 24 hours.
- For longer storage, up to 7 days, preserve the sample with 2 mL concentrated sulfuric acid per liter and store at 4°C.

Note, when samples are preserved with acid, the test result will include nitrite and nitrate.

METHODS SUMMARY

- For measuring nitrate in water we can use a simple procedure (HACH DR2000 spectrophotometer and HACH Nitrate pillows).
- REPORTING Report the nitrate concentration of the raw wastewater and the treated water as required according to the Sampling and Analysis Plan in the Monthly Report. Results should be reported to the nearest one tenth (for example, 0.5 mg/L).
- QUALITY CONTROL Results can be controlled by analyzing a duplicate sample and standard analysis.

Refer to the Procedures Manual Method No. 13

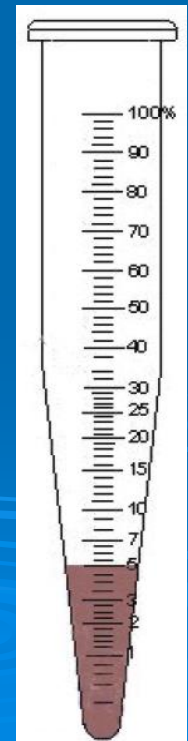
14- CENTRIFUGE TEST

SCOPE AND APPLICATION

- The solids inventory is a very important measure to control the Activated Sludge process. Any excess sludge must be wasted to maintain the Mixed Liquor Suspended Solids (MLSS) within the target range (is process dependent and usually found in the design criteria section of the O & M manual).

Total Solids (% by Volume)

The centrifuge compresses the sludge sample into the bottom of the conical shaped test tube. Graduations on the tube indicate the percent of total volume in the tube occupied by solids.



EQUIPMENTS

RAVEN F-10300 PROCESS CENTRIFUGE

- Six-position rotor with 19mm Centrifuge Tubes rotates at a right angle to the axis of spin and provides a sharp, easily read solids/liquid interface.
- Timer (with fifteen minute speed key) to start and automatically switch unit OFF at end of test.
- Corrosion resistant stainless steel and aluminum construction for durability and easy cleaning.
- 1/8 HP, 3000 RPM, totally enclosed fan-cooled motor.



B-10101-19 Centrifuge Tubes (19mm)



➤ Sample Collection

Collect at least 50 ml. of sample for the centrifuge spin in a wide mouth container. If the Settleometer Test is being performed in conjunction with the centrifuge test, the centrifuge sample can be taken from the same mixed liquor container.

➤ Mix sample

The sample to be poured into the centrifuge tube should be gently mixed (but not shaken).

➤ Fill a clean tube with sample, so that the bottom of the meniscus is at the 100% mark on the tube. Samples should be poured quickly to prevent settling in the sample collection container.

➤ Detailed Centrifuge Test Procedure

Refer to the Procedures Manual Method No. 14

Spin Test



NOTE

- Immediately switch [OFF] the centrifuge in the event of an unbalanced condition. (ON/OFF switch is at rear of unit near power cord)
- Do not open lid while centrifuge is spinning.
- Do not move unit while operating.
- Unplug power cord from centrifuge before opening lid.

➤ **Clean sample tubes**

- The centrifuge tubes must be thoroughly cleaned prior to reuse or future results will be inaccurate. Cleaning is best achieved by forcing a high velocity stream of tap water into the bottom of the emptied tube to dislodge the solids.

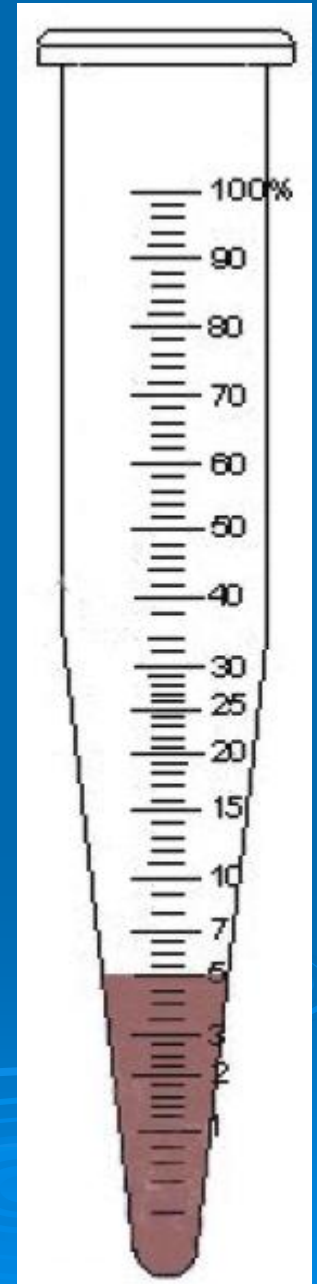
Test Results

Total Solids concentration can be read in percent volume.

This value should not be converted to mg/l concentration because of variations in density and compactibility of different types and ages of sludge samples.

Typical ranges are:

1. Mixed Liquor: 0.5-5%
2. Return and Waste Sludge : 3 to 20%
 - a. Young (bulking sludge): <10%
 - b. Normal: 10-20%
 - c. Old (denitrifying sludge): >20%
3. Gravity thickened waste activated sludge: 5 to 30%



Solids Weight-to-Concentration Ratio (WCR)

➤ EXAMPLE:

For a mixed liquor sample determine the Mixed Liquor Suspended Solids (MLSS) (see method No 7) and spin the same sample in the centrifuge to determine the Aeration Tank Concentration (ATC) by volume.

MLSS = 3000 mg./L.

ATC = 3%

CALCULATE: $WCR = MLSS/ATC$

$WCR = 3000 / 3 = 1000 \text{ mg/L/\%}$

WCR varies among different plants and other factors may influence this index. Unusual amounts of grit may increase WCR dramatically. WCR is a density index since it is a weight divided by a volume.

➤ TYPICAL WEIGHT-TO-CONCENTRATION RATIOS

OLD SLUDGE	WCR=>1000
NORMAL SLUDGE	WCR=500-1000
YOUNG SLUDGE	WCR=<500

15- MICROSCOPIC EXAMINATION OF ACTIVATED SLUDGE

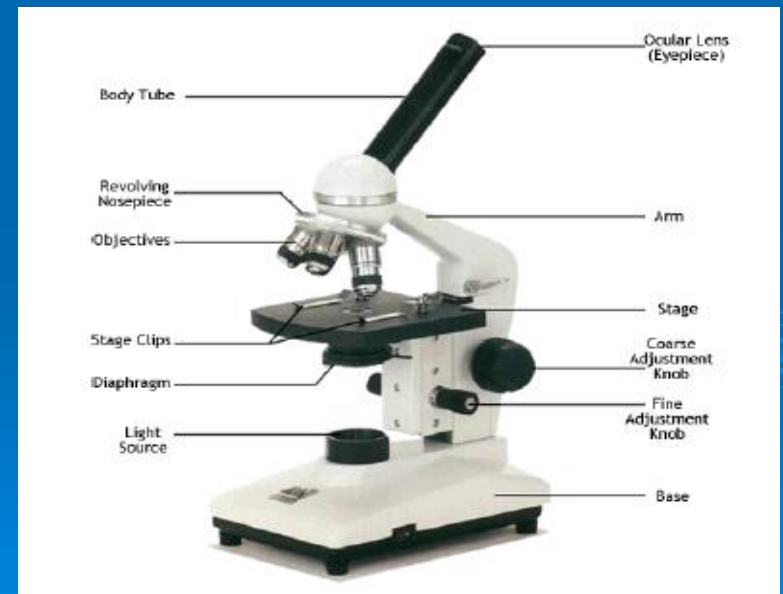
Microscope

Frequent microscopic examination can provide valuable information about the microbiological composition and condition of the activated sludge.

Observing and recording the different types of microorganisms can help the wastewater treatment plant operator identify changes that may cause a plant upset.

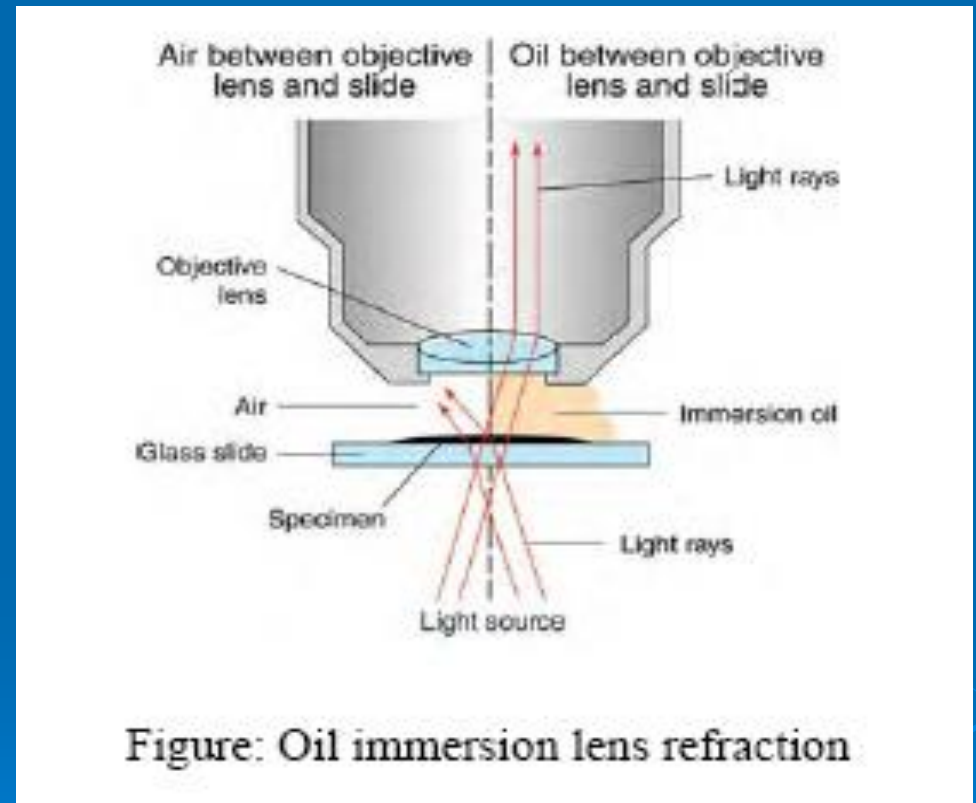
Parts of a Microscope

- The eyepiece or ocular lens is located at the top of the microscope. It may be monocular or binocular. The eyepiece usually magnifies the object 10x.
 - Objective lenses are mounted on a turret or nosepiece. There will be either 3 or 4 objective lenses. The objective lenses are rotated and come in close contact with the microscope stage.
- The 10x, 20x or 40x objectives are all used to see the object in more detail.*



Oil immersion lens.

- The 100x objective lens is called an oil immersion lens. This lens provides a magnification of 1000 times. Oil is used to reduce light diffraction and improve the resolution,
- The oil should always be cleaned from the lens after each use to reduce the accumulation of dirt on the lens

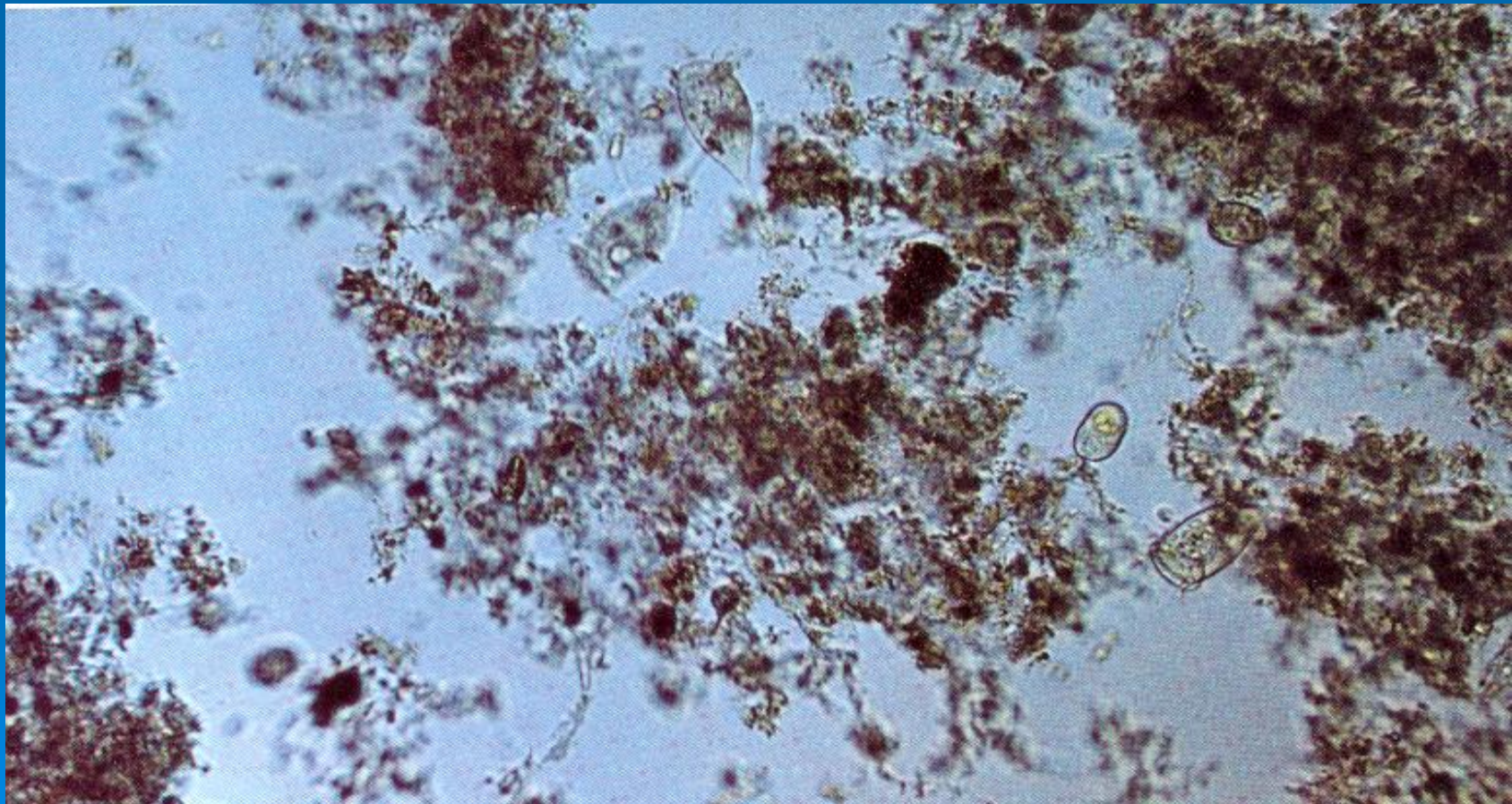
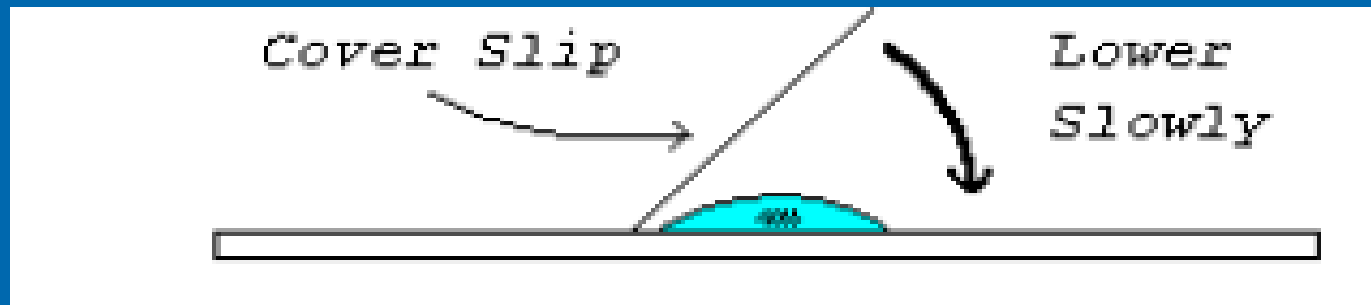


The microscope stage

- The microscope stage is the platform that holds the slide.
- A good microscope will have two slide movement knobs. These knobs allow the slide to be slowly moved back and forth on the stage.
- Below the stage are the diaphragm lever and the condenser.
- The condenser consists of a series of lenses which focus the light on the slide.
- The condenser is almost always kept close to the stage for maximum image sharpness.

Lens Cleaning

- It is critical to keep all lenses clean, including the condenser.
- Use only lint-free cleaning tissues and store in a dust free environment with the dust cover on.
- Objective lenses should be cleaned using manufacturer approved solvents. The most common approved solvent is xylene.



Common Microorganisms



Bacillus bacteria

Spiral Bacteria

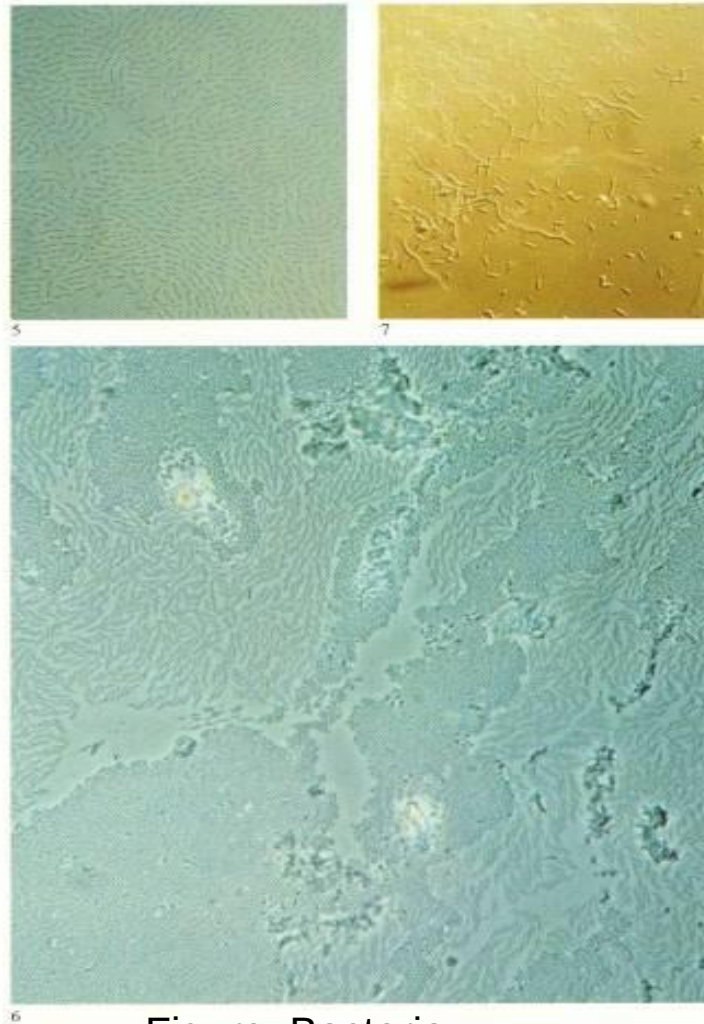
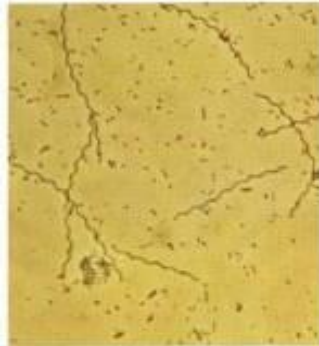


Figure: Bacteria

Vitreoscilla



8



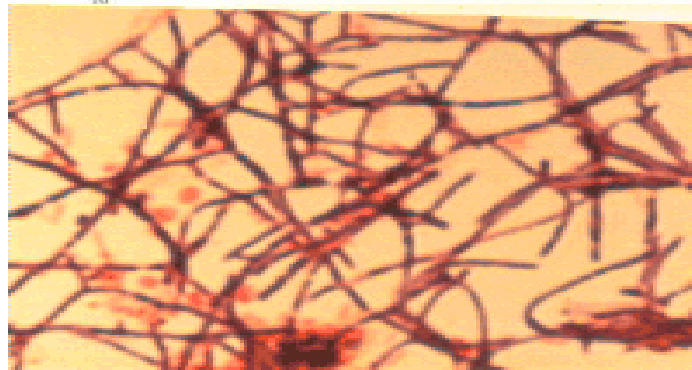
9

Sphaerotilus



10

Beggiatoa



11

Filamentous Bacteria

1

Protozoans

- Protozoa are single-celled, animal like organisms which include amoebas, flagellates, ciliates, and sporozoans. Protozoa can be free-living or parasitic. Common parasitic protozoa found in water cause amoebic dysentery Giardiasis, and Cryptosporidiasis.

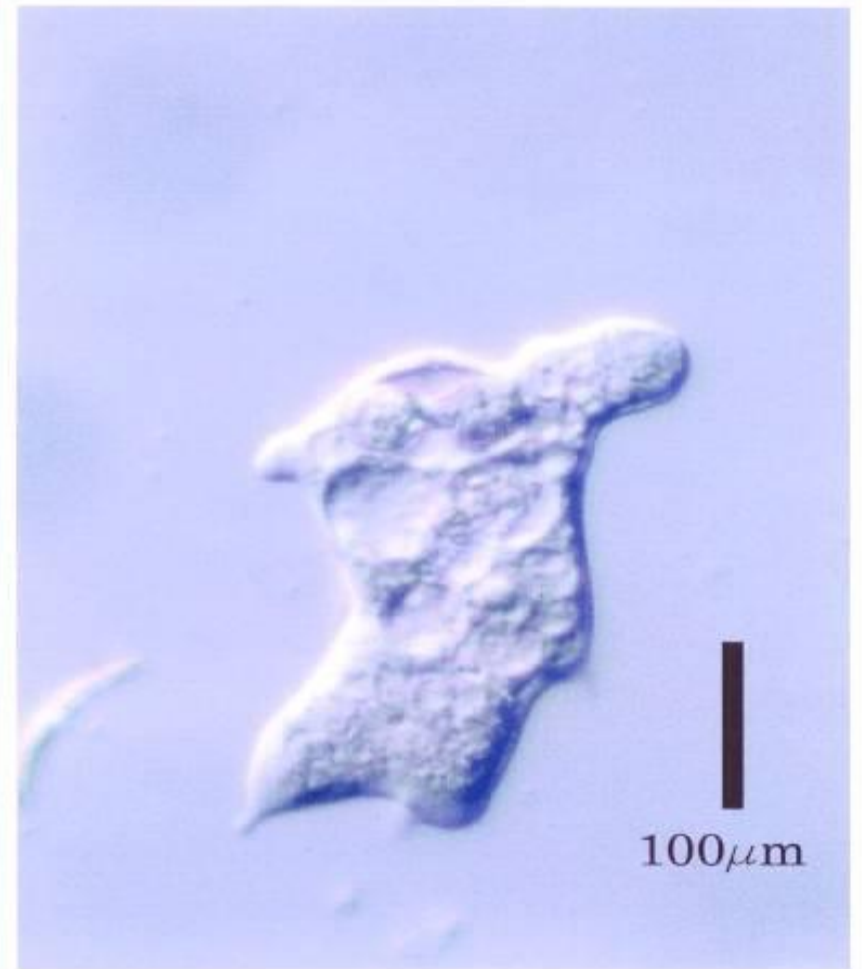
Amoeba



Amoeba



Testate Amoeba



Flagellates



Free-swimming ciliates

- The presence of free-swimmers indicate the activated sludge system is approaching optimum treatment conditions. The presence of lots of grazers is more positive than the presence of cruisers.

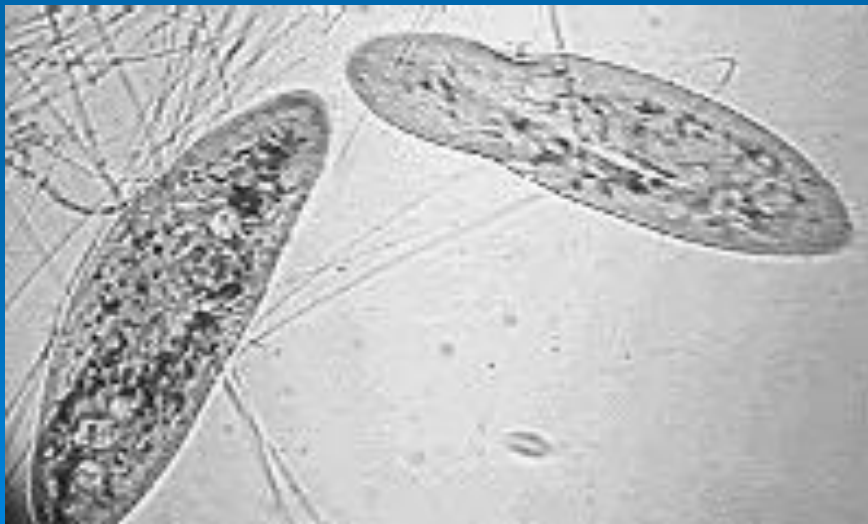


Figure: Grazing ciliate

Stalked ciliates

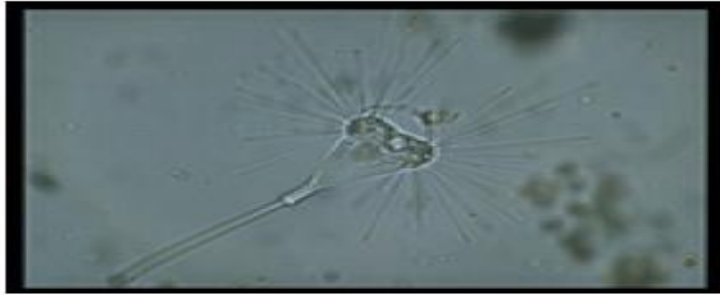


Figure: Stalked ciliate

- Stalked ciliates may occur individually or form colonies. They begin to predominate when free swimming ciliates are unable to compete for food.
- When stalked ciliates are present, floc formation is usually good and the effluent is clear. Ciliates are easily visible under 100x and 200x magnification

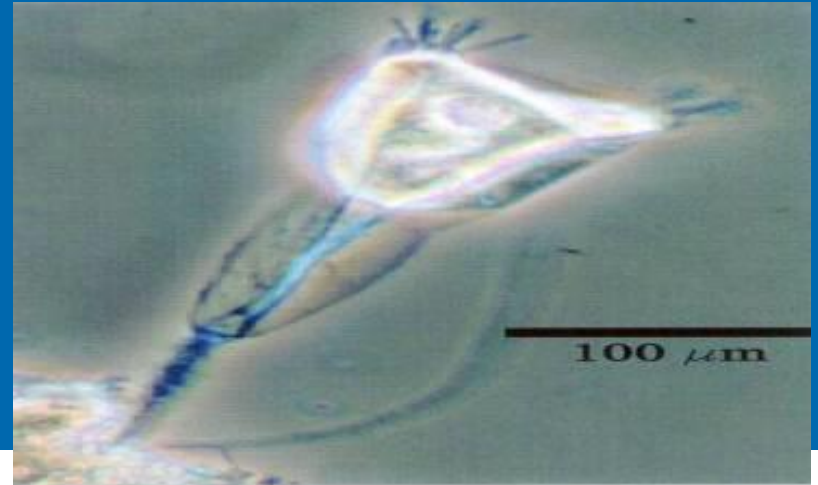


Figure: Colonized Stalked Ciliates



Vorticella convallaria

Carchesium



Metazoa

Metazoa are the largest organisms in activated sludge systems. They include rotifers, nematodes, and bristleworms.

Rotifers

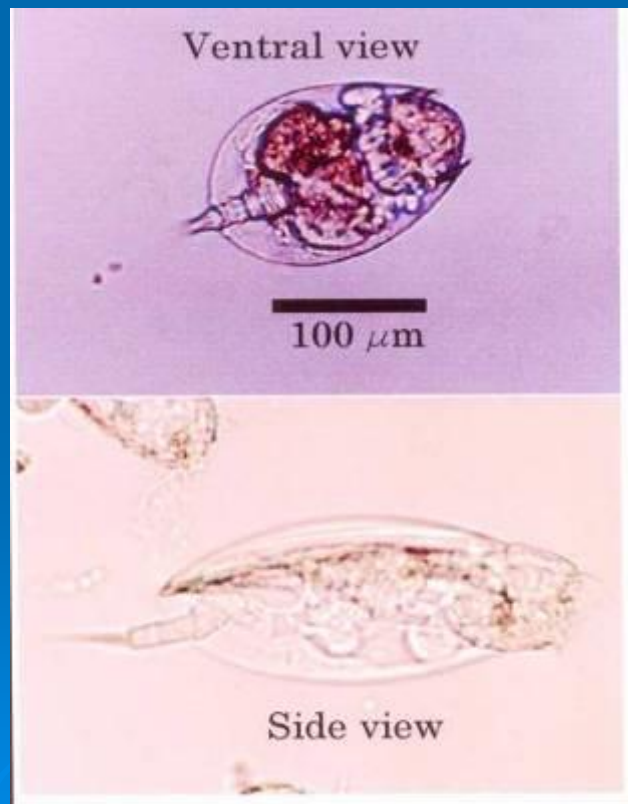
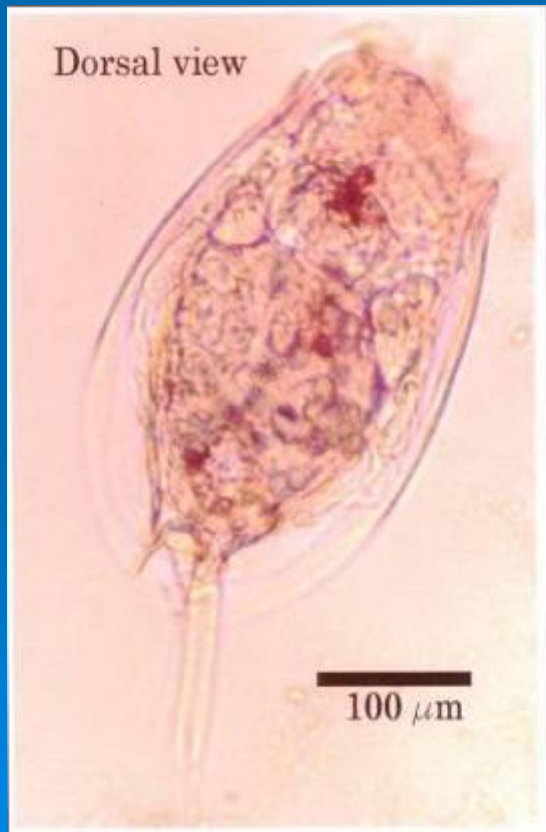


Figure: Rotifer

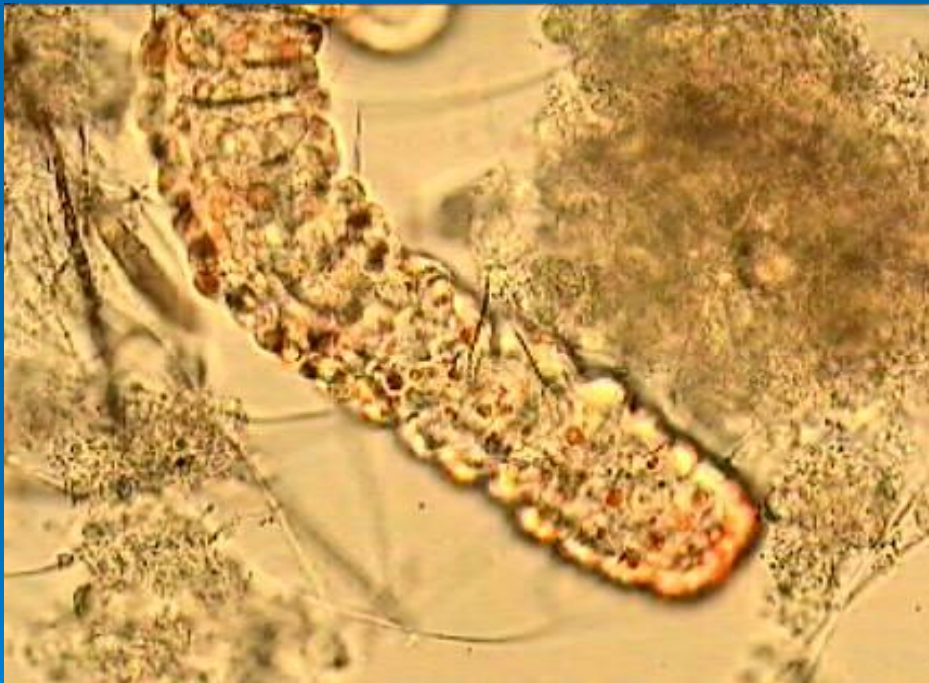
Nematodes

- Nematodes commonly found in wastewater are non-segmented roundworms. They have a long, slender body with one end usually sharply pointed while the other end tapers to a blunt tip (little fire hoses).
- They feed on bacteria, protozoa, ciliates, rotifers, and other bacteria composing the floc.
- The presence of nematodes indicates an older sludge. Sludge worms are easily seen under a 4X magnifying glass.



Bristleworms

Aeolosoma is the segmented worm commonly found in very old activated sludge. It has bristles which extend from its sides and burnt orange dots on its surface. Bristleworms live of the organic sludge.



16- AMMONIA NITROGEN NH_3 - N SPECTROPHOTOMETER DR 2000 Method 4500- NH_3 .

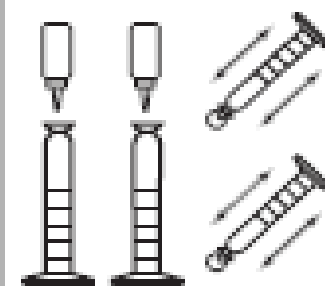
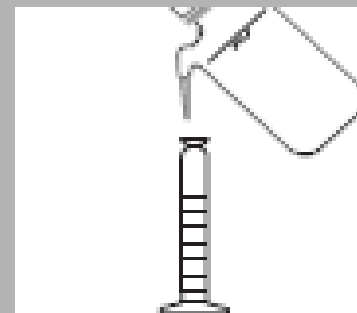
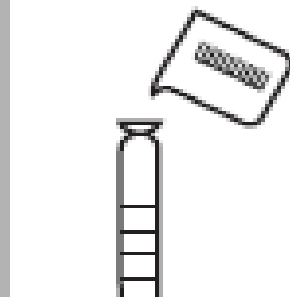
- **Principle of Method:** use of the spectrophotometer DR2000
- **Scope and Application:** range 0.02 – 2.5 mg/l NH_3 , (no sample distillation required)
- **Interferences:** amines, mercury, and silver
- **Sample preservation:** analyze as soon as possible, if not preserve with 0.8 ml of conc. sulfuric acid per liter of sample; before analysis neutralize again to pH = 7 with 5N sodium hydroxide

DR 2000 PROCEDURE



Method 8038

Hach Programs



1. Touch

Hach Programs.

Select program

380 N, Ammonia, Ness.

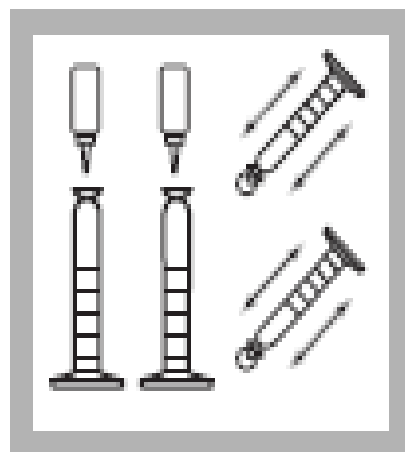
Touch Start.

2. Fill a 25-mL mixing graduated cylinder to the 25-mL mark with sample (this is the prepared sample).

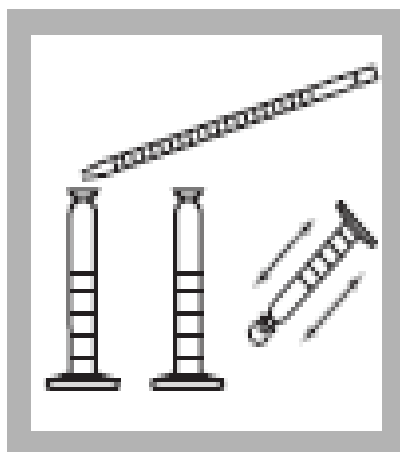
3. Fill a 25-mL mixing graduated cylinder to the 25-mL mark with deionized water (this is the blank).

4. Add three drops of Mineral Stabilizer to each cylinder.

Stopper and invert several times to mix.



5. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder.
Stopper and invert several times to mix.



6. Pipet 1.0 mL of Nessler Reagent into each cylinder.
Stopper and invert several times to mix.



7. Touch the timer icon.
Touch OK.
A one-minute reaction period will begin.



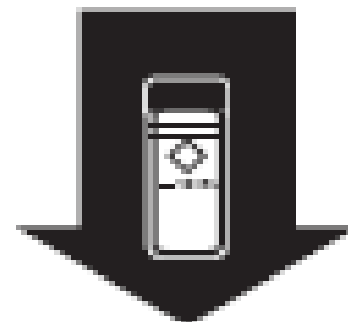
8. Pour each solution into a round sample cell.



9. When the timer beeps, place the blank into the cell holder.

Zero

10. Touch Zero.
The display will show:
0.00 mg/L $\text{NH}_3\text{-N}$



11. Wipe the prepared sample and place it into the cell holder.
Results will appear in
mg/L $\text{NH}_3\text{-N}$.

Interferences

Interfering Substance	Interference Levels and Treatments
Chlorine	Remove residual chlorine by adding 2 drops of sodium arsenite for each mg/L Cl from a 250 mL sample. Sodium thiosulfate can be used in place of sodium arsenite. See <i>Sample Collection, Storage, and Preservation</i> below.
Hardness	A solution containing a mixture of 500 mg/L CaCO_3 and 500 mg/L Mg as CaCO_3 does not interfere. If the hardness concentration exceeds these concentrations, add extra Mineral Stabilizer.
Iron	Interferes at all levels by causing turbidity with Nessler Reagent.
Seawater	May be analyzed by adding of 1.0 mL (27 drops) of Mineral Stabilizer to the sample before analysis. This complexes the high magnesium concentrations found in sea water, but the sensitivity of the test is reduced by 30 percent due to the high chloride concentration. For best results, perform a calibration, using standards spiked to the equivalent chloride concentration, or distill the sample as described below.
Sulfide	Interferes at all levels by causing turbidity with Nessler Reagent.
Glycine, various aliphatic and aromatic amines, organic chloramines, acetone, aldehydes and alcohols	May cause greenish or other off colors or turbidity. Distill the sample if these compounds are present.

Using a Hach Spectrophotometer DR 2000

- **Refer to the Procedures Manual Method No.16**

17- 1 BIOCHEMICAL OXYGEN DEMAND

BOD₅

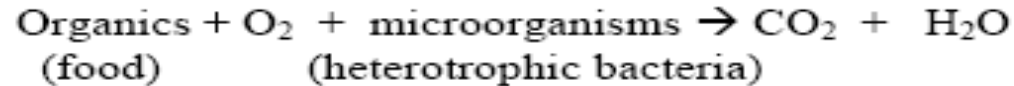
INTRODUCTION

- BOD is a measure of oxygen consumed by microorganisms under specific conditions.
- BOD is used to evaluate the biodegradability of waste water and the residual oxygen demand of the effluent once discharged.
- To calculate the organic loading on various process units

Biological Oxygen Demand

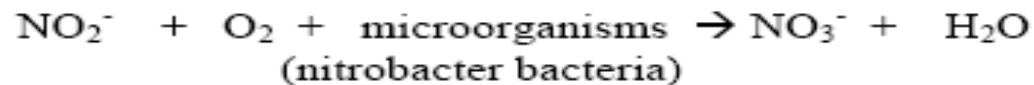
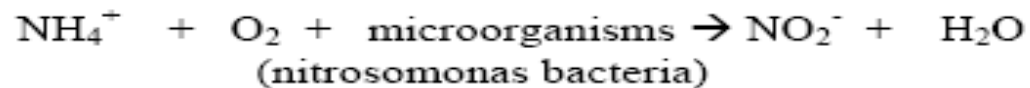
1. Oxygen is consumed by microorganisms as they biochemically degrade (oxidize) organic materials (*carbonaceous demand*)

Reaction 1



2. Oxygen is consumed by microorganisms to oxidize reduced forms of nitrogen such as ammonium or nitrite. (nitrogenous demand)

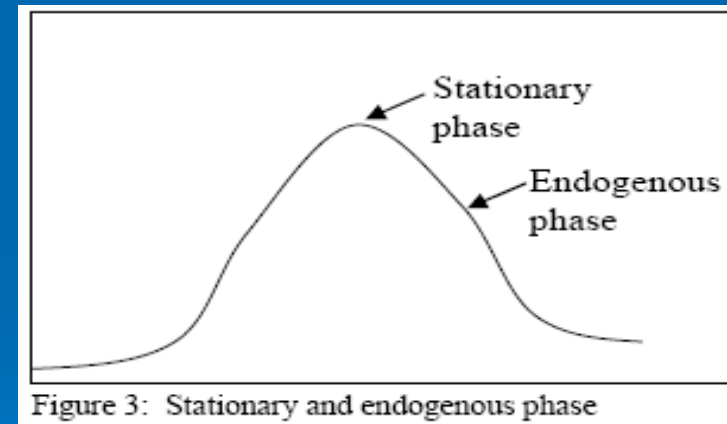
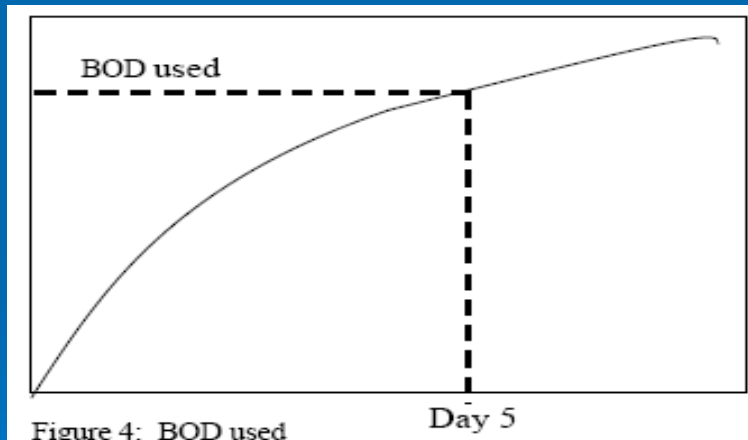
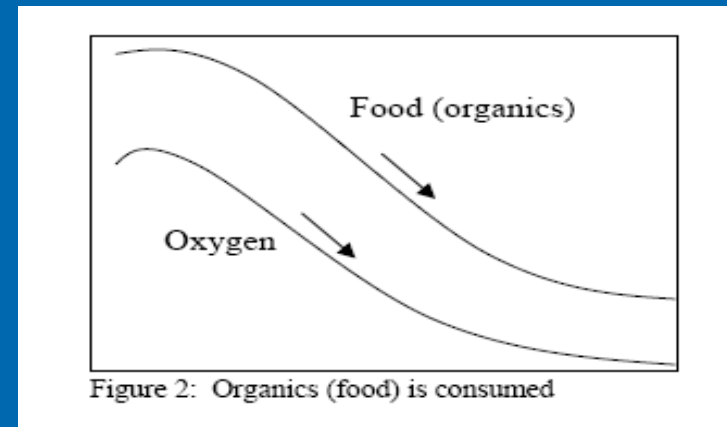
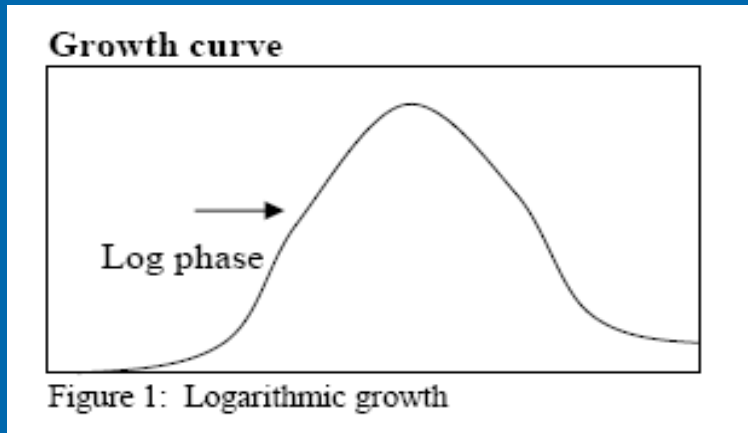
Reaction 2



3. Oxygen is consumed when reduced inorganic compounds such as sulfides and ferrous iron are present. This often occurs in septic sewer lines.



BOD Test



Complete biological oxidation of a sample may require a period of incubation too long for practical purposes. Five days has been selected as the standard incubation period for historic reasons (time for waste discharge to the Thames River in England to reach the ocean).

BOD₅, CBOD and NBOD

BOD Procedure

Refer to the Procedures Manual Method no.17

Dilution Requirements

Seed Control

How many bacteria are needed?

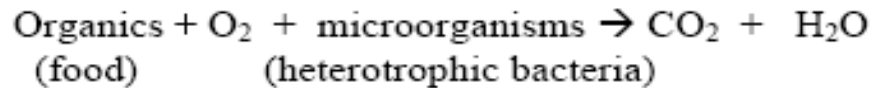


Figure 5.

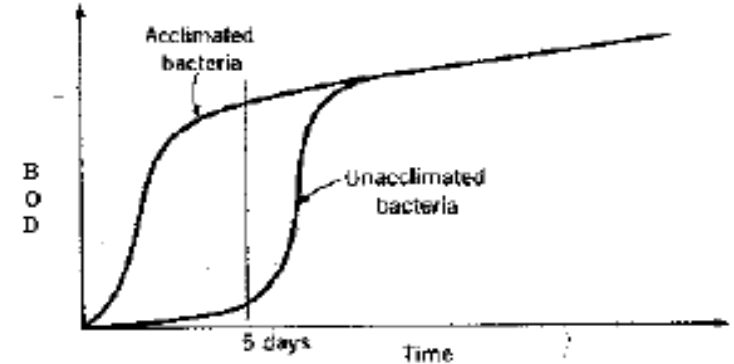
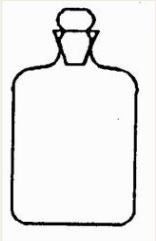
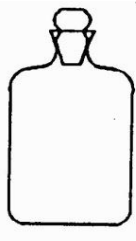
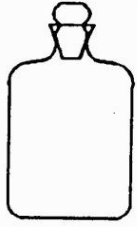
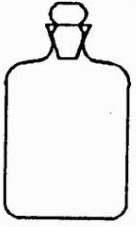
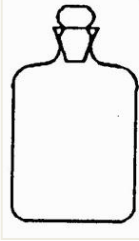
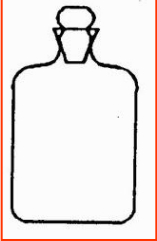
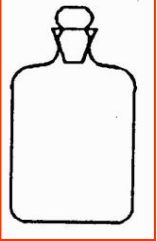
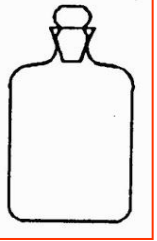
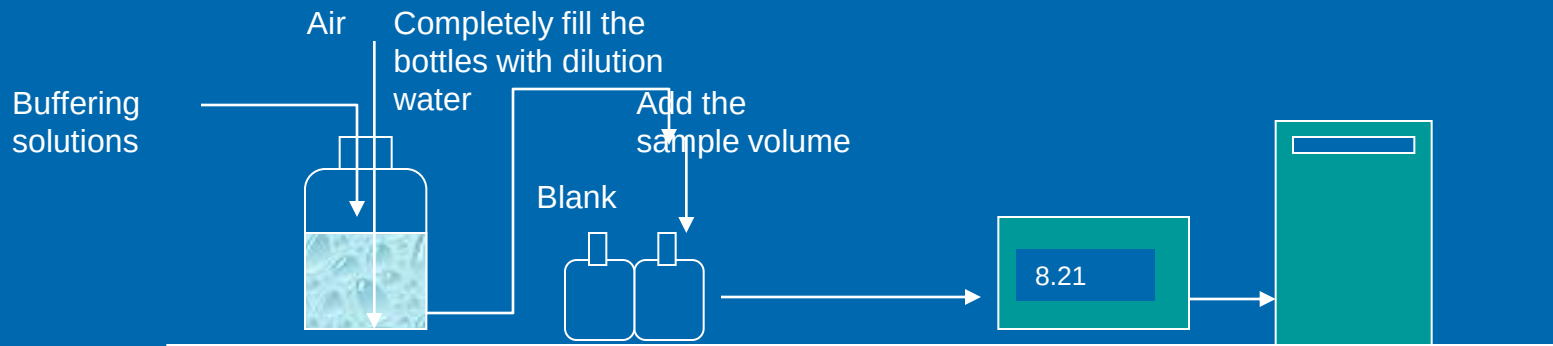


Figure 5: Acclimated seed

BOD BOTTLE ARRANGEMENT (D.O.METER)

Initial DO (4 Bottles)					
5 Day DO (4 Bottles)					
	Blank		1 st Dilution	2 nd Dilution	3 rd Dilution

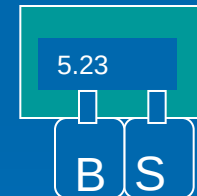


Prepare the aerated dilution water

Fill the BOD bottles

Measure the initial DO

Incubate for 5 days at 20 °C



Measure the 5th day DO

EXAMPLE:

BOD Bottle Volume (V)	= 300 mL
Sample Volume	= 15 mL
Initial DO of Diluted Sample (A)	= 8.0 mg/L
DO of diluted sample after 5-day incubation (B)	= 4.0 mg/L

CALCULATION:

$$\text{BOD5 mg/l} = \frac{(A - B) \times V}{\text{Sample volume}}$$

$$\text{BOD5 mg/l} = \frac{(8.0 - 4.0) \times 300}{15} = 80 \text{ mg/l}$$

Seed Correction Calculation

- To make this correction, seed correction bottles are used.
- The seed correction bottles determine how much of the oxygen used over 5 days was due to the seed itself.

sample organics + O₂ + seed —————→ BOD1
seed organics + O₂ + seed —————→ BOD2

- The BOD test will measure **BOD1 + BOD2**
- The BOD caused by the seed organics must be subtracted or "corrected".

Seed Correction Calculation

Example

if 10 ml of seed is added to a BOD bottle, filled with dilution water, then incubated for 5 days.

- Initial Dissolved Oxygen = 7.7 mg/L
- Final Dissolved Oxygen = 5.4 mg/L
- Dissolved oxygen used = 2.3 mg/L

$$\frac{2.3\text{mg} / l}{10\text{ml}} = \frac{0.23\text{mg} / l}{1\text{ml of seed}}$$

- Each milliliter of seed used 0.23 mg/L of oxygen over the 5 days. Two milliliters of seed would have used 0.46 mg/L of oxygen. Three milliliters of seed would use 0.69 mg/L of oxygen, etc.
- This test suggests to the analyst to add enough seed to the diluted sample so that each standard or sample bottle have a seed correction of 0.6-1.0 mg/L. By targeting this range, there should be ample microorganisms present to oxidize the organics in the sample

QUALITY CONTROL

Glucose - Glutamic Acid (GGA) Standard

the BOD should be 198 ± 30.5 mg/L. Results outside this range indicate a problem that requires immediate correction and invalidates all samples that were also run.

Example 1:

- Calculate the sample BOD concentration for a chlorinated WWTP effluent sample with an expected BOD of 20 mg/L.
- The seed correction was 0.3 mg/L per ml of seed.
- In this example, three different volumes of sample were placed in the BOD bottles, 30, 60, and 100 ml. The sample volumes selected were based on the anticipated result. Standard Methods recommends preparing enough bottles so that at least 2 of the bottles will meet the DO depletion rule.

BOD bottle number	6	3	51
Volume of sample added	30	60	100
Volume of seed added	2	2	2
Initial DO	7.5	7.5	7.4
Final DO	3.9	1.1	0.0
DO Difference	3.6	6.4	
Seed Correction	0.6	0.6	
Corrected Difference	3.0	5.8	
BOD	30.0	29.0	

WASTEWATER TREATMENT PLANT

BOD ₅ WORK SHEET											SAMPLE DATE: / /			ANALYSIS DATE: /		
20 th Edition, <i>Standard Methods for the Examination of Water and Wastewater</i>						TIME:			0 DAY DO DATE: /							
PROCEDURE NUMBER 5210 B						TIME:			5 DAY DO DATE: /							
8:00 AM INCUBATOR TEMPERATURE, °C DO1																
8:00 AM INCUBATOR TEMPERATURE, °C DO5						ANALYST:										
(1) SAMPLE TYPE/ID	Raw		Eff		Blank											
(2) BOTTLE NUMBER	1	2	3	4	9											
(3) SAMPLE VOLUME, mL	2	5	10	15	300											
(4) DILUTION FACTOR = 300 / (3)	150	60	30	20	1											
(5) INITIAL DO, mg/L	8.12	8.12	8.11	8.10	8.13											
(6) 5- DAY DO, mg/L *	6.54	4.02	7.34	7.12	8.10											
(7) DO DEPLETION, mg/L = (5) - (6) **	1.58	4.10	0.77	0.98	0.03											
(8) BLANK DEPLETION CORRECTION, mg/L = [(7) of Blank, mg/L X DW, mL] / 300	0.0298	0.0295	0.0290	0.0285												
(9) DO CORRECTION = (7) - (8)	1.55	4.07	0.74	0.95												
(10) BOD ₅ , mg/L = (9) X (4)	232.53	244.23	22.23	19.03												
(11) BOD ₅ AVERAGE	238.38		20.63													
* The residual 5-day-DO must be at least 1mg/L																
** 5-day-DO uptake 2 mg/L or greater																
*** No depletion correction needed if DO depletion in the blank is less than 0.2 mg/l																
DW = Dilution Water																

REMEMBER

- The temperature of the incubator must be at 20°C. Other temperatures will cause different bio-kinetics (change of rate of oxygen used).
- The dilution water must be prepared according to Standard Methods to have the most favorable conditions for bacteria to grow. This water must be free of copper which is often present as copper distills are used by commercial dealers. Use all glass or stainless steel distills or de-mineralized water.
- The wastewater must also be free of toxic waste, such as hexavalent chromium.
- If you use a cleaning solution to wash BOD bottles, be sure to rinse the bottles several times. Cleaning agents can be toxic and if any residue remains in a BOD bottle the BOD test could be ruined.

BOD Troubleshooting

The BOD test is invalid when

1. BOD incubator temperature is outside $20 \pm 1^\circ\text{C}$ or
2. The GGA standard is outside $198 \pm 30.5 \text{ mg/l}$

Other anomalies during the test occurred.

Troubleshooting BOD Dilution Water:

If the dilution water blanks drop more than 0.2 mg/L , the dilution water needs to be evaluated. The BOD test is not invalid. However, the analyst must begin to seek a remedy for the problem. Possible problems include:

- 1. Poor aeration procedure. (Common error)
- 2. The dissolved oxygen meter was improperly calibrated. (Common error)
- 3. Bottle filling was too vigorous (air bubbles got trapped)
- 4. Dilution water temperature was too cold.
- 5. Dirty BOD bottles and/or stoppers. It's easy to forget to scrub the stoppers (Common error)
- 6. The nutrient reagents were contaminated.
- 7. De-ionized water source was contaminated.

17-2 Respirometric Method

➤ The method uses pressure measurements via piezo-resistive electronic pressure sensors.

➤ **BOD₅ Determination**

The wastewater usually contains enough nutrient salts and suitable microorganisms. Under these conditions the BOD₅ determination with the respirometric system is possible in the undiluted sample.

Required instruments and accessories

OxiTop Measuring System.

- Inductive stirring system.
- Incubator to maintain the temperature at 20°C for 5 days.
- Sample bottles 510 ml.
- Stirring rod remover.
- Suitable overflow beakers.
- Rubber quiver.
- Sodium hydroxide tablets.

Selection of sample volume

- Estimate the BOD₅ value to be expected for the wastewater sample.
- Look for the corresponding measuring range in the following table and gather correct values for the sample volume and factor.



Sample volumes and factors, corresponding to the expected BOD5.

Sample volume (ml)	Measuring range mg/l	Factor
432	0 – 40	1
365	0 – 80	2
250	0 – 200	5
164	0 – 400	10
97	0 – 800	20
43.5	0 – 2000	50
22.7	0 - 4000	100

•PROCEDURE

Refer to the Procedures Manual Method No.17-2

CALCULATION

Convert the displayed measured value (digits) into a BOD₅ value with the table using the following formula:

$$\text{Digits} \times \text{Factor} = \text{BOD}_5 \text{ mg/l}$$

18- CHEMICAL OXYGEN DEMAND (COD)

INTRODUCTION

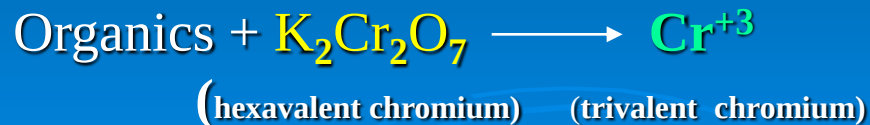
- The COD (Chemical Oxygen Demand) test is used to measure the content of chemically oxidizable organics using a strong oxidizing agent (usually potassium dichromate) in an acidic medium at an elevated temperature.
- Most permits specify BOD₅ because it gives a better idea of how the organics are affecting the receiving stream (consume oxygen).
- COD has the advantage over BOD₅ in that the analysis can be completed within a few hours whereas BOD₅ requires 5 days. The major drawback of the COD test is the use of hazardous chemicals and their appropriate disposal.
- COD test yields better reproducible results whereas BOD₅ is more susceptible to human error.

Importance of COD Measurement

- COD is used as a global parameter of organic pollutants in wastewater and natural waters.
- In domestic wastewater BOD_5 represents about 40-60% of the COD. The presence of toxic materials or industrial wastewater can alter this ratio.

COD Theory

- Like in the BOD test oxygen is used to oxidize the organics to carbon dioxide and water. Oxygen is chemically bound in the oxidizing agent $K_2Cr_2O_7$ (**potassium dichromate**).
- As the potassium dichromate is used the reduced Cr^{+3} ion is produced. The amount of dichromate used is proportional to the amount of organics present. Likewise, the amount of Cr^{+3} ion present is proportional to the amount of organics oxidized.



Closed Reflux, Colorimetric Method

Refer to the Procedures Manual Method No.18

- This method uses test tubes with pre-measured amounts of potassium dichromate, sulfuric acid, and a catalyst.

For the digestion to occur, the reaction needs acid, heat, and a catalyst.

- The sample will get very hot when the sample is added to the acid and mixed. Be sure the cap is on tight before mixing and mix just prior to placing in the digestion reactor.
- The catalyst used is silver. However, silver must be soluble and will precipitate if chlorides are present in the sample. To prevent silver precipitation, mercury has been added to the reagents in the tube. The mercury will remove the chloride interference.
- Once the 2 hour refluxing period is finished, the remaining potassium dichromate is measured using the spectrophotometer.
- A reagent blank and standard must be prepared to calibrate the spectrophotometer to zero

COD REACTOR AND SPECTROPHOTOMETER



SAFETY: Be very careful when adding water to acid. This is contrary to normal safety procedure. Be sure to wear goggles, gloves, apron, etc. and do not mix until the cap is tightened on the test tube.



The heat will be provided by the digestion reactor which is set at 150°C. The sample is refluxed (digested) for 2 hours. During the 2 hours, the organics are oxidized by the acid, potassium dichromate and catalyst.



Sample Collection

- The sample is usually collected the same way as the BOD₅ sample
- The COD sample may be a composite or a grab sample.
- Because there are no microorganisms involved in this procedure, preservation is usually done by acidification using sulfuric acid to a pH below 2. The sample holding time for COD analysis is 7 days, much longer than the 24 hours allowed for the BOD₅ test.

Sample Handling

- The sample volume used for the COD test is 2.0 ml so measuring the sample volume is critical.
- Be sure to mix the sample well and homogenize if necessary. Pipet quickly to avoid settling errors.
- COD can be run on industrial samples that may have high BOD₅. (If the COD strength is greater than 1500 mg/L, the sample must be diluted.)

Select the Method

Hach Chemical Co. uses two methods for measurement of COD

(Refer to the Procedures Manual Method No.18

- a high range 0-1500 mg/L and
- a low range, 0-150.

Use the low range for effluent samples and the high range for all other samples. The procedure for both methods is essentially the same until measuring with the spectrophotometer.

The concentration of dichromate in the low level method is 10x lower than in the high level method.

- KHP Standard Preparation
- Sample Digestion
- Sample Measurement

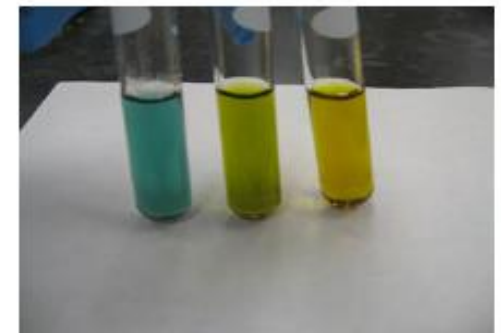
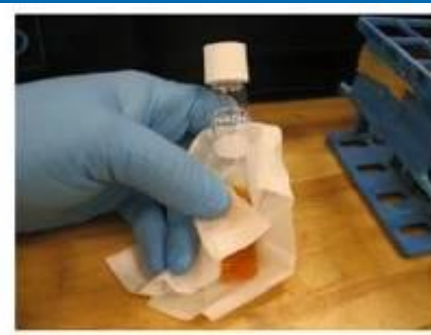


Figure: Sample dilutions. Left sample is >1500 mg/L. All dichromate is gone.

Troubleshooting

- Test is usually trouble free. Most errors occur with the blank and KHP standard.
 - The spectrophotometer must be warmed up and zeroed using the reagent blank. If the volume of DI water added to the reagent blank is not 2.0 ml, the color of the reagent blank will be either lighter or darker than expected. This error will be most noticeable when using the low range method.
 - If the KHP standard does not fall into the range of 500 ± 50 , all samples should be invalidated. The lab technician should evaluate the Standard preparation procedure.
 1. Was the balance calibrated prior to weighting the KHP?
 2. Was the Standard weighed correctly?
 3. Was there any powder spilled during the Standard preparation?
 4. Was the KHP volume pipetted correctly?
 5. Were the caps on the Standard loose during digestion?
 6. Were the sample cells wiped clean?
 7. Is the correct wave length being used?
 8. Has the KHP Standard deteriorated? (eg. cloudy appearance)
- If the KHP Standard and the digestion reactor temperature were incorrect, the data must be invalidated.

WASTEWATER TREATMENT PLANT LABORATORY												
COD WORK SHEET							SAMPLE DATE: / ANALYSIS DATE: / /					
20th EDITION, of <i>The Standard Method for the Examination of Water and Wastewater</i> PROCEDURE NUMBER 5220 D CLOSED REFLUX, COLORIMETRIC							TIME:					
(1) SAMPLE TYPE	Blank e	Blanke	KHF 500	Inf HR	Inf HR	Eff LR	Eff LR	Raw HR	Raw HR			
(2) BOTTLE NUMBER	1	2	3	4	5	6	7	8	9			
(3) SAMPLE VOLUME, ml			2	2	2	2	2	1	1			
(4) DILUTION FACTOR = 2 / (3)			1	1	1	1	1	2	2			
(5) COLORIMETER READING mg/l			520	1320	1340	12	15	920	895			
(6) COD mg/l =(Sample(5) - Blank(5))x (4)			520	1320	1340	12	15	1840	1790			
AVERAGE			520	1330		13.5		1815				

19- TOTAL COLIFORMS

Introduction

- Coliforms found in water or sludge are an indicator for the presence of pathogenic bacteria. If Coliforms are absent, the water or sludge is assumed to be safe.
- Total Coliform count can be used as a measure of the bacteriological quality. This parameter is principally determined in the effluent

TEST METHODS

Total Coliform bacterial numbers may be determined either by:

- the Multiple-tube procedure (not further explained here)
- the Membrane Filter (MF) technique.

Refer to the Procedures Manual Method No.19

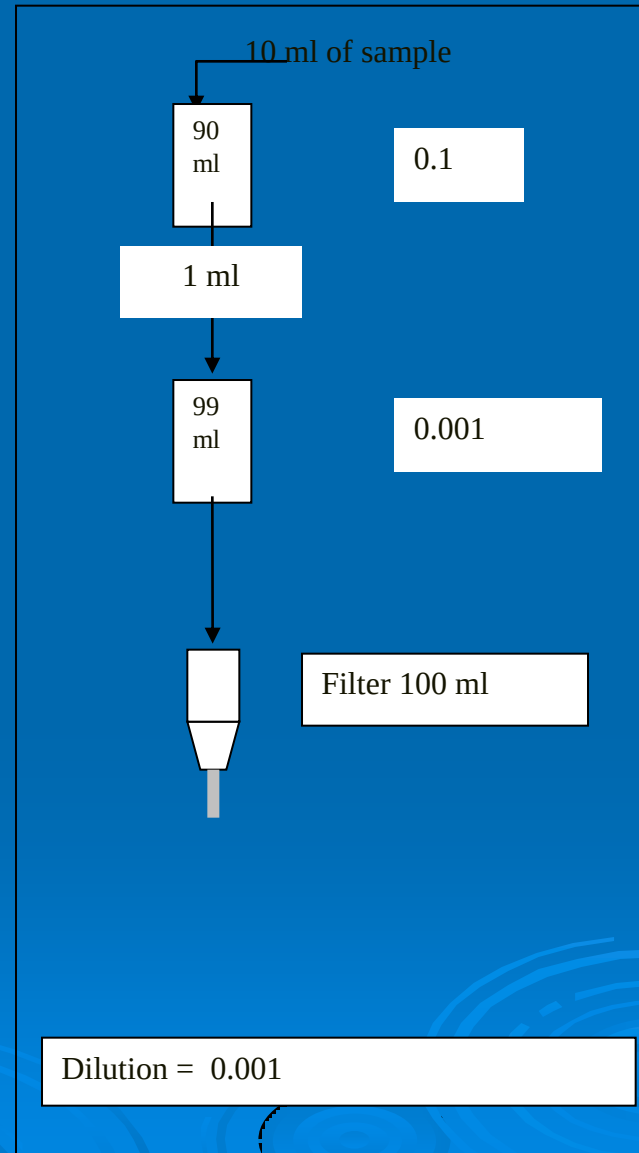
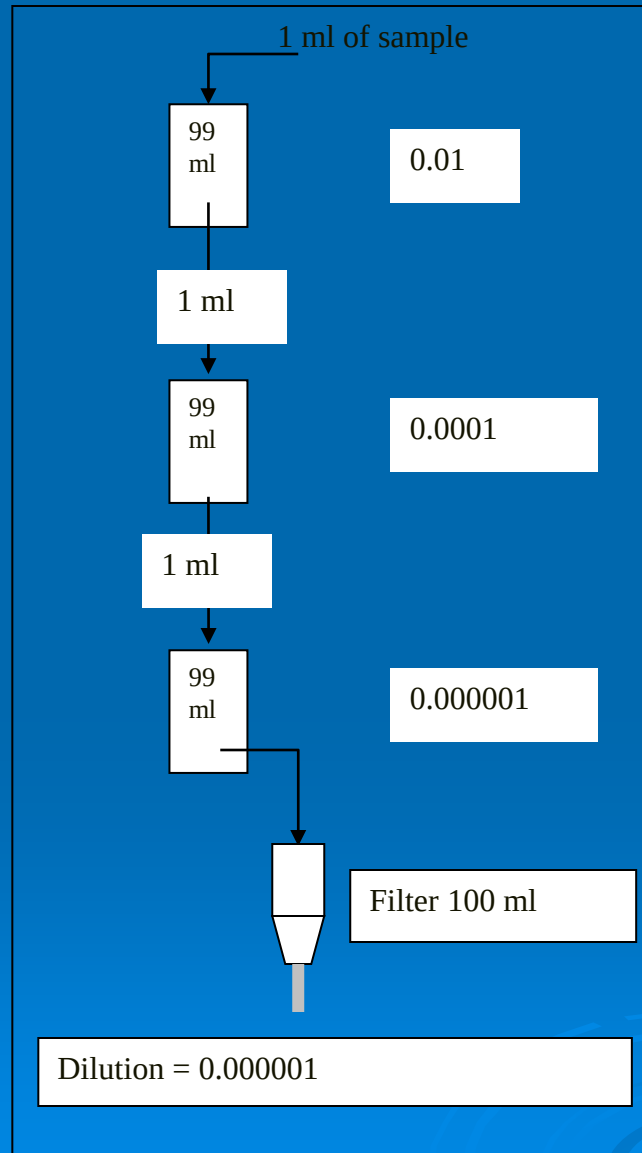
The Membrane Filter (MF) technique.

- The Total Coliform MF procedure uses an enriched lactose medium and incubation temperature of $35.5 \pm 0.2^{\circ}\text{C}$ for selectivity.
- Because incubation temperature is critical, use an appropriate incubator that is documented to hold the 35.5°C temperature within 0.2°C throughout the chamber, over a 24-h period.
- Sample bottles
- Dilution bottles
- Pipets and graduated cylinders
- Culture dishes
- Incubator
- M-ENDO medium

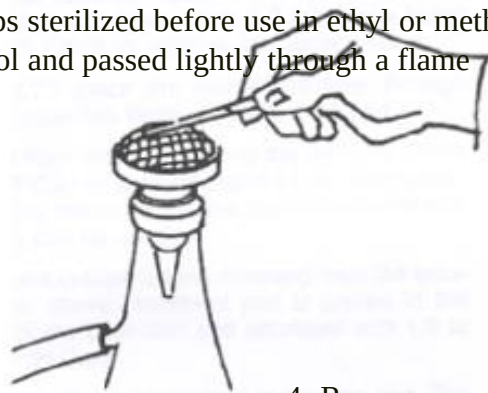
Counting:

- Colonies produced by Total Coliform bacteria on M-ENDO medium are **red with a metallic sheen**.
- Non-Total Coliform colonies are dark brown.
- Normally, few non-Total Coliform colonies will be observed on M-FC medium because of selective action at the elevated temperature and the selectivity of the media.
- Count colonies with a low-power (10 to 15 magnifications) binocular wide-field dissecting microscope or other optical device.
- Select a volume of the wastewater sample to be examined (about 50-100 ml). Use sample volume and dilution that will yield counts between 20 and 60 Total Coliform colonies per membrane.
- When the bacterial density of the sample is unknown, filter several volumes or dilutions to achieve a countable density. Estimate volume and/or dilution expected to yield a countable density and select two other volumes (usually one-tenth and ten times the original volume).

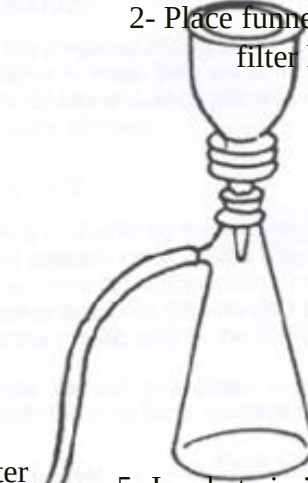
Dilution scheme



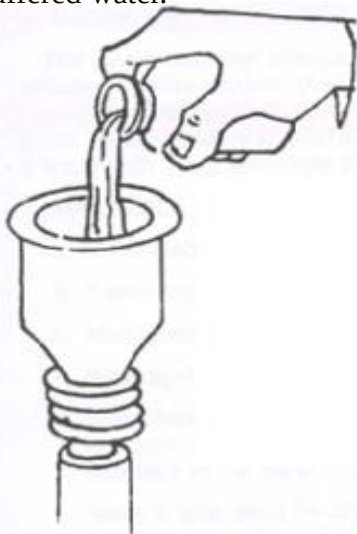
1-Center membrane filter on filter holder.
Handle membrane only on outer 5mm with
forceps sterilized before use in ethyl or methyl
alcohol and passed lightly through a flame



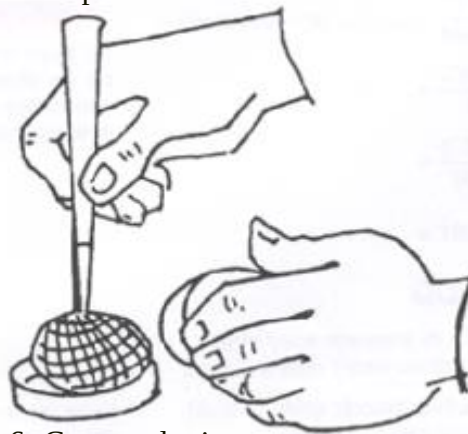
2- Place funnel onto
filter holder



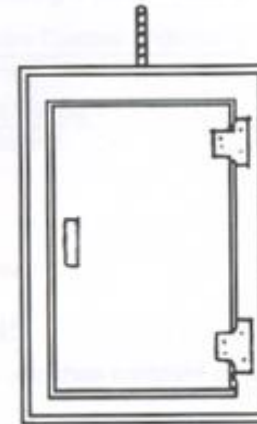
3- Pour sample aliquot into funnel.
Avoid spattering. After suction is
applied, rinse three times with sterile
buffered water.



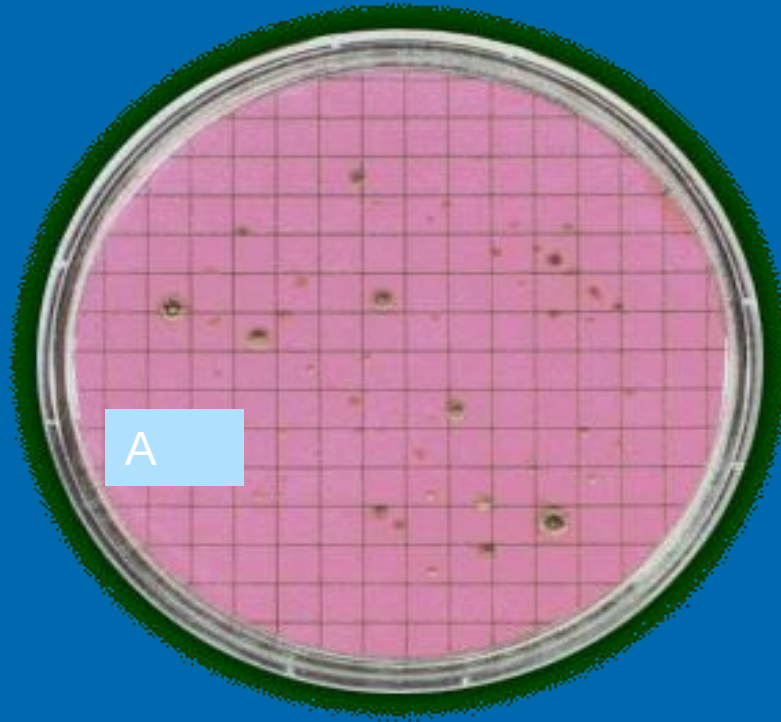
4- Remove membrane filter
from filter holder with sterile
forceps. Place membrane on
pad with media. Cover with
Petri dish top



5- Incubate in inverted
position for 24 hours at 35.5
°C



6- Count colonies on
membrane



EXAPMLES OF TOTAL COLIFORM PLATE COUNTS

A -plate shows the metallic sheen colonies of Total Coliforms growing on M-ENDO media. The number of colonies as shown is perfect for counting. The sample size and dilution used are correct.



B – plate shows no colonies growing on M-ENDO media. The sample collected from filtered chlorinated water.

C – plate shows a large number of Total Coliform colonies due to incorrect dilution (this sample can not be counted effectively).

CALCULATION

$$\text{Bacteria/100 mL} = \frac{\text{No. of Colonies Counted}}{\text{Dilution factor}}$$

OR

$$\text{Bacteria/100 mL} = \frac{\text{No. of Colonies Counted} \times 100 \text{ mL}}{\text{Sample Volume Filtered, mL in 100 ml}}$$

EXAMPLE 1 :

A total of 42 colonies grew after filtering 100 ml of a sample with dilution factor 1: 10.

$$\text{Bacteria/100 mL} = \frac{42}{0.1} = 420/100 \text{ ml}$$

EXAMPLE2:

A total of 22 colonies grew after filtering a 10-mL sample.

$$\begin{aligned} &= \frac{(22 \text{ colonies})(100 \text{ mL})}{(10 \text{ mL})} \\ &= 220 \text{ per 100 mL} \end{aligned}$$