

ENVIRONMENTAL ENGINEERING LABORATORY

LAB MANUAL

Year : 2019 - 2020
Course Code : ACE112
Regulations : IARE – R16
Semester : VII
Branch : CE

Prepared By

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DEPARTMENT OF CIVIL ENGINEERING

INSTITUTE OF AERONAUTICAL ENGINEERING

(Autonomous)

Dundigal, Hyderabad - 500 043



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DEPARTMENT OF CIVIL ENGINEERING

Program: Bachelor of Technology (B. Tech)

VISION OF THE DEPARTMENT

To produce eminent, competitive and dedicated civil engineers by imparting latest technical skills and ethical values to empower the students to play a key role in the planning and execution of infrastructural & developmental activities of the nation.

MISSION OF THE DEPARTMENT

To provide exceptional education in civil engineering through quality teaching, state-of-the-art facilities and dynamic guidance to produce civil engineering graduates, who are professionally excellent to face complex technical challenges with creativity, leadership, ethics and social consciousness



INSTITUTE OF AERONAUTICAL ENGINEERING

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DEPARTMENT OF CIVIL ENGINEERING

Program: Bachelor of Technology (B. Tech)

PROGRAM OUTCOMES (PO's)	
PO1	Engineering knowledge: Apply the knowledge of mathematics, science, engineering fundamentals, and an engineering specialization to the solution of complex engineering problems.
PO2	Problem analysis: Identify, formulate, review research literature, and analyze complex engineering problems reaching substantiated conclusions using first principles of mathematics, natural sciences, and engineering sciences
PO3	Design/development of solutions: Design solutions for complex engineering problems and design system components or processes that meet the specified needs with appropriate consideration for the public health and safety, and the cultural, societal, and environmental considerations.
PO4	Conduct investigations of complex problems: Use research-based knowledge and research methods including design of experiments, analysis and interpretation of data, and synthesis of the information to provide valid conclusions.
PO5	Modern tool usage: Create, select, and apply appropriate techniques, resources, and modern engineering and IT tools including prediction and modeling to complex engineering activities with an understanding of the limitations.
PO6	The engineer and society: Apply reasoning informed by the contextual knowledge to assess societal, health, safety, legal and cultural issues and the consequent responsibilities relevant to the professional engineering practice.
PO7	Environment and sustainability: Understand the impact of the professional engineering solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.

PO8	Ethics: Apply ethical principles and commit to professional ethics and responsibilities and norms of the engineering practice.
PO9	Individual and team work: Function effectively as an individual, and as a member or leader in diverse teams, and in multidisciplinary settings.
PO10	Communication: Communicate effectively on complex engineering activities with the engineering community and with society at large, such as, being able to comprehend and write effective reports and design documentation, make effective presentations, and give and receive clear instructions.
PO11	Project management and finance: Demonstrate knowledge and understanding of the engineering and management principles and apply these to one's own work, as a member and leader in a team, to manage projects and in multidisciplinary environments.
PO12	Life-long learning: Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change.

ENVIRONMENTAL ENGINEERING LABORATORY – SYLLABUS

Exp. No.	Name of the Experiment
1.	Determination of pH and Turbidity
2.	Determination of Conductivity and Total Dissolved Solids (Organic and Inorganic)
3.	Determination of Alkalinity/Acidity
4.	Determination of Chlorine
5.	Determination of Iron
6.	Determination of Dissolved Oxygen
7.	Determination of Nitrates
8.	Determination of Optimum Dose of Coagulant
9.	Determination of Chlorine Demand
10.	Determination of Total Phosphorous
11.	Determination of B.O.D
12.	Determination of C.O.D
13.	Test for Coliforms in water

ATTAINMENT OF PROGRAM OUTCOMES (PO's) & PROGRAM SPECIFIC OUTCOMES (PSO's)

ACE112 - Environmental engineering Lab	Program Outcomes	Program Specific Outcomes
Extrapolate the knowledge of preparation of PH in water	PO 1, PO 7	PSO1
Use innovative methods to improve the quality of soft water for industrial purpose at cheaper cost.	PO 1,PO 4,PO 7	PSO1
Evaluate conductometry and conductometric titrations	PO 1	PSO1
Estimate potentiometric and potentiometric titrations.	PO 1	PSO1
Compare the results of experiments with potentiometer	PO 1	PSO1
Describe PH in water	PO 1,PO 4,PO 7	PSO1
Identify the formula for dissolved oxygen	PO 1,PO 7	PSO1
Explain certain properties of water using the concepts of alkalinity and acidity	PO 1,PO 4,PO 7	PSO1
Develop theoretical aquatic chemistry basis and use the principles for the evaluation of water quality.	PO 1,PO 7	PSO1
Describe the rate constant for a reaction and elementary steps in the reaction mechanism.	PO 1	PSO1
Explore the basic knowledge of adsorption.	PO 1	PSO1
Understand principles and their practical application chromatographic separation	PO 1	PSO1

MANDATORY INSTRUCTIONS

1. Students should report to the labs concerned as per the timetable.
2. Record should be updated from time to time and the previous experiment must be signed by the faculty in charge concerned before attending the lab.
3. Students who turn up late to the labs will in no case be permitted to perform the experiment scheduled for the day.
4. After completion of the experiment, certification of the staff in-charge concerned in the observation book is necessary.
5. Students should bring a notebook of about 100 pages and should enter the readings/observations/results into the notebook while performing the experiment.
6. The record of observations along with the detailed experimental procedure of the experiment performed in the immediate previous session should be submitted and certified by the staff member in-charge.
7. Not more than FIVE students in a group are permitted to perform the experiment on a set up.
8. The group-wise division made in the beginning should be adhered to, and no mix up of student among different groups will be permitted later.
9. The components required pertaining to the experiment should be collected from Lab- in-charge after duly filling in the requisition form. When the experiment is completed, students should disconnect the setup made by them, and should return all the components/instruments taken for the purpose.
10. Any damage of the equipment or burnout of components will be viewed seriously either by putting penalty or by dismissing the total group of students from the lab for the semester/year.
11. Students should be present in the labs for the total scheduled duration.
12. Students are expected to prepare thoroughly to perform the experiment before coming to Laboratory.

13. Procedure sheets/data sheets provided to the students groups should be maintained neatly and are to be returned after the experiment.
14. DRESS CODE:
 1. Boys - Formal dress with tuck in and shoes.
 2. Girls - Formal dress (salwarkameez).
 3. Wearing of jeans is strictly prohibited

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Experiment No-1

1a. Determination of pH of Water

Aim

To determine the pH of given samples using

- (1) Universal indicator
- (2) pH paper, and
- (3) Digital pH meter.

Principle

pH value of water indicates the hydrogen ion concentration in water and concept of pH was put forward by Sorenson (1909). pH is expressed as the logarithm of the reciprocal of the hydrogen ion concentration in moles/ litre at a given temperature. The pH scale extends from 0 (very acidic) to 14 (very alkaline) with 7 corresponding to exact neutrality at 25°C. pH is used in the calculation of carbonate, bicarbonate and CO₂, corrosion and stability index etc. While the alkalinity or acidity measures the total resistance to the pH change or buffering capacity, the pH gives the hydrogen ion activity. pH can be measured colorimetrically or electrometrically.

Colorimetric method is used only for rough estimation. It can be done either by using universal indicator or by using pH paper. The hydrogen electrode is the absolute standard for the measurement of pH. They range from portable battery operated units to highly precise instruments. But glass electrode is less subjected to interferences and is used in combination with a calomel reference electrode. This system is based on the fact that a change of 1 pH unit produces an electric charge of 59.1 mV at 25°C.

Apparatus

1. pH meter with electrode
2. Beaker
3. Thermometer
4. Colour comparator with discs
5. Cuvettes

Reagents

1. Buffer solutions
2. pH paper
3. Universal indicator

Procedure

(a) Using Universal Indicator

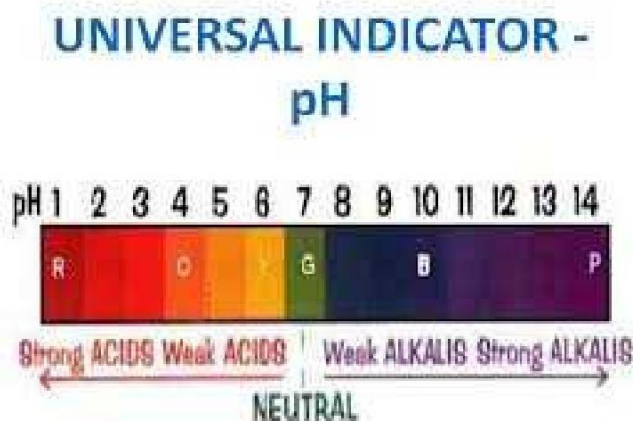
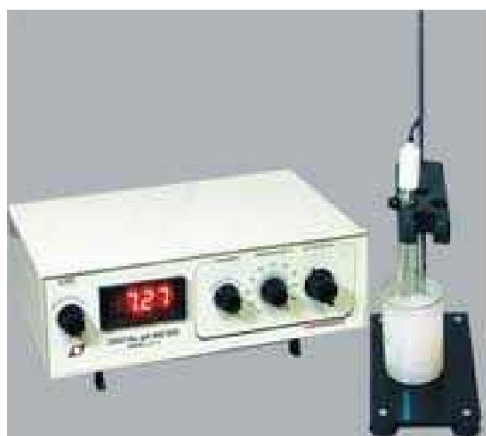
(If comparators are not available, compare the colour with colours given in the chart.)

1. **Using pH Papers** 10 mL of sample is taken in a cuvette.
 2. Another 10 mL sample is taken in another cuvette and 0.2 mL of universal indicator is added and placed in the hole provided for.
 3. A colour disc corresponding to this indicator is inserted into the comparator and the disc rotated such that the 2 circles indicate identical colours.
 4. The reading is noted.
 5. The procedure can be repeated using an indicator whose range is near the value obtained.
 6. The exact pH is obtained.
-
1. Dip the pH paper in the sample.

2. Dip the pH paper in the sample.
2. Compare the colour with that of the colour given on the wrapper of the pH paper book.
3. Note down the pH of the sample along with its temperature.

(c) Using pH Meter

1. Follow the manufacturer's operating instructions.
2. Dip the electrode in the buffer solution of known pH.
3. Switch on the power supply and take the reading. Standardize the instrument using the calibrating knob.
4. After cleaning, again dip the electrodes in the buffer solution of pH 7. Note the reading. If it is 7, the instrument is calibrated. If not, correct the value and is manipulated so that the reading in the dial comes to 7.0.
5. A solution whose pH is to be found is taken in a beaker and the temperature knob is adjusted such that the temperature of solution is same as that in dial.
6. The electrode is washed with distilled water and reused with the solution and then it is dipped in the solution.
7. The reading on the dial indicates the pH of the solution.



Results:

Sample no.	pH		
	pH paper	pH meter	Universal indicator
1			
2			
3			

Experiment No-1

1b. Determination of Turbidity

Aim :

To determine the turbidity of the given sample using Nephelometer in NTU.

Principle

The method presented below is based on a comparison of the intensity of light scattered by the sample in specific conditions with the intensity of light scattered by standard reference suspension under the same condition. The higher the intensity of scattered lights, higher the turbidity. Formazine polymer, which has gained acceptance as the turbidity standard reference suspension is used as a reference turbidity standard suspension for water. It is easy to prepare and is more reproducible in its lights scattering properties than the clay or turbid natural water standards previously used. The turbidity of a given concentration of formazine has an approximate turbidity of 100 NTU, when measured on candle turbidity meter. Nephelometric turbidity units based on formazine preparation will have approximate units derived from Jackson candle turbidimeter but will not be identical to them.

Apparatus

Nephelometer with accessories

Reagents

- (i) Turbidity free distilled water (for setting zero).
- (ii) Formazine turbidity concentrate (hydrazine sulphate + hexamine).
- (iii) Formazine standard (for setting 100 of the instrument).



Nephelometer

Preparation of Turbidity Free Distilled Water

Pass distilled water through a membrane filter having a precision pore size of less than 10 microns (What man filter No. 42). Rinse collecting flask atleast twice with such filtered water and discard the next 200 mL. Use this filtered water for setting zero of the instrument.

Preparation of Formazine Turbidity Concentrate

(a) Solution I

Weigh accurately 5 g of 'Anal-R' quality hydrazine sulphate $(\text{NH}_2)_2\text{H}_2\text{SO}_4$ into a 500 mL volumetric flask and add distilled water to make up to the mark. Leave the mixture to stand for 4 hours.

(b) Solution II

Weigh accurately 50g of 'Anal-R' quality hexamethylenetetramine (CH_2N_4) (hexamine) into a 500 mL volumetric flask and add distilled water to make up to the mark. Mix equal volume of solution I and II to form formazine turbidity concentrate. Allow it to stand in a closed container at 25°C to 30°C for 48 hours to produce insoluble white turbidity corresponding to 4000 NTU.

Note: Once prepared, formazine turbidity concentrate (which corresponds to 10000 ppm SiO_2) is stable for 2 to 3 months.

Preparation of Formazine Standard

Dilute 25mL of the formazine turbidity concentrate to 1 litre with turbidity free distilled water to obtain 250 ppm or 100 NTU for setting '100' of the instrument.

Note: Formazine standard 100 NTU should be prepared weekly.

Procedure

- (1) Switch the instrument on.
- (2) Open the lid of the sample compartment.
- (3) Insert a test tube filled with distilled water into the sample compartment.
Close the lid.
- (4) Adjust 'SET 0' control to get '0' displayed on the read out.
- (5) Open the lid. Replace the test tube filled with distilled water with a test tube filled with formazine standard. Close the lid.
- (6) Adjust the 'SET 100' control to get '100' displayed on the read out.
- (7) Repeat the above operation to get consistent values of 0 to 100 within 1% to 2%.

Measurement of turbidity less than 100 NTU

1. Thoroughly shake the sample.
2. Wait until air bubbles disappear and pour the sample into the nephelometer tube.

3. Read the turbidity directly from the instrument.

Measurement of turbidity above 100 NTU

Dilute the sample with one or more volume of turbidity free distilled water until the turbidity fall below 100 NTU.

$$\text{NTU of sample} = \frac{A(B+C)}{C}$$

A = NTU found in diluted sample

B = volume of dilution water in mL

C = sample volume taken for dilution in mL

Observation:

<i>0-100 NTU</i>					<i>> 100 NTU</i>		
<i>Sample No.</i>		<i>NTU</i>	<i>A</i> <i>mL</i>	<i>B</i> <i>mL</i>	<i>C</i> <i>mL</i>		<i>NTU = A(B=C)/C</i>

Results :

<i>Description of Sample</i>	<i>Turbidity in NTU</i>

Viva Questions:

1. What is the significance of PH in environmental engineering?
2. What are the types of water can be used to find out PH values?
3. What are the standard results of pH?

Experiment No-2

2a. Determination of Conductivity

Conductivity is a numerical expression of the ability of an aqueous solution to carry the electric current. This ability depends on the presence of ions, their mobility, valence, relative concentrations and on the temperature of measurement. The inorganic acids, bases, and salt solutions are relatively good conductors. On the contrary, molecules of organic compounds that do not dissociate in aqueous solution have a poor conductivity.

The conductivity is measured in the laboratory in term of resistance measured in ohms. The electric resistance of a conductor is inversely proportional to its cross sectional area and directly proportional to its length. The magnitude of the resistance measured in an aqueous solution therefore depends on the characteristics of the conductivity cell used. Specific resistance is the resistance of a cube of 1cm. In aqueous solutions such a measurement is seldom made because of the difficulties in fabrication of electrode. Actually the electrodes measure a given fraction of the specific resistance known as the cell constant C

$$C = \frac{\text{Measured resistance, } R_m}{\text{Specific resistance, } R_s}$$

The reciprocal of resistance is conductance. It measures the ability to conduct a current and is expressed in reciprocal of ohms i.e mhos. In water analysis generally micromhos is used. Knowing the cell constant the measured conductance is converted to the specific conductance or conductivity, K_s , as the reciprocal of the specific resistance.

$$K_s = 1/R_s = C/R_m$$

The term conductivity is preferred and usually reported in micromhos per centimeter (μ mhos/cm)

Freshly made distilled water has a conductivity of 0.5 to 2.0 μ mhos/cm that increases

after some days due to the absorption of CO₂ from atmosphere.

The conductivity of potable waters varies generally from 50 to 1500 μ mhos/cm. The conductivity of municipal waste waters may be near to that of the potable water.

However 10000 μ mhos/cm.

Measurement of conductivity with lesser accuracy than laboratory analysis is done continuously by the field recorders. These automatic recorders give idea about any sudden drastic change in the quality of raw water or the waste water, so that required precautions may be taken.

Actually the total dissolved solids in water can be estimated by measuring its conductivity and multiplying it by an empirical factor. This factor varies from 0.55 to 0.9 depending upon the soluble components of water and the temperature. This factor can be obtained for a system by observing the conductivity and the dissolved solids and then it can be used for continuous monitoring.

Apparatus

- (a) **Conductivity meter:** This is an instrument consisting of a source of alternating current, a Wheatstone bridge, a null indicator and a conductivity cell. Generally an instrument capable of measuring conductivity with an accuracy of 1 % or 1 μ mhos/cm is used. A thermometer capable of reading upto 0.1o C within a Range of 15 to 30°C is used.
- (b) **Conductivity Cell:** Platinum-electrode type conductivity cells containing platinized electrodes are used depending upon the expected range of conductivity. Non platinum-electrode type conductivity cells containing electrodes constructed from durable metals like stainless steel are used for continuous monitoring systems.

Reagents

- (a) **Conductivity water:** Pass distilled water through a mixed bed deionizer and discard first liter. Conductivity should be less than 1 μ mhos/cm mg.
- (b) **Standard Potassium Chloride Solution (KCl, 0.01M),** Dissolve 745.6 of anhydrous KCl in conductivity water and dilute to 1000 ml at 25oC. This the tandard reference solution

having a conductivity of 1413 $\mu\text{mhos/cm}$ at 25°C , useful for the cell constants between 1 and 2.

Procedure

(i) Determination of Cell Constant

Wash the conductivity cell with 0.01 M KCl solution. Adjust the temperature of the standard KCl at $25 \pm 0.1^\circ\text{C}$. Measure resistance of the KCl and note the temperature.

The Cell Constant, $C = (0.001413) (R_{\text{KCL}}) [1 + 0.0191(t - 25)]$

(ii) Conductivity Measurement

Rinse cell with the sample. Adjust temperature of the sample to $25 \pm 0.1^\circ\text{C}$. Measure sample resistance or conductivity and the temperature

If the temperature deviates from 25°C the corrected conductivity shall be as follows

$$K = \frac{(K_m) C}{(1 + 0.019(t - 25))}$$

K_m is the measured conductivity at $t^\circ\text{C}$.



OBSERVATIONS AND CALCULATION

Water sample no.	Temperature	Electrical conductivity μ mhos / cm	Total dissolved solids in mg/l= EC x 'K' (selecte or measured 'K')

Result

The electrical conductivity of the given water sample is μ mhos/ cm

Viva Questions:

1. What is the significance of conductivity?
2. What is the standard value for conductometer?

Experiment No-2

2b. Determination of Total Dissolved Solids

Theory :

Sewage contains 99.9% water and only 0.1% solids but the nuisance caused by them is considerable, as they are highly putrescible (readily degradable) and therefore require proper treatment before disposal. The solids present in sewage may be classified as suspended and dissolved solids which may further be subdivided into volatile and non volatile solids. The volatile matter is organic matter. Quantification of volatile or organic fraction of solid which is putrescible is necessary as this constitutes the load on biological treatment units or oxygen resources of a stream when sewage is disposed of in a river. The dissolved solid may be inorganic also and the inorganic fraction is considered when sewage is used for land irrigation or when reuse of sewage is done for any other purpose. The measurement of total dissolved solids in water can be done in similar way, by taking the sample of water, in place of sewage.

Apparatus

- (i) Evaporating dishes
- (ii) Drying oven
- (iii) Standard filter paper
- (iv) Digital weighing balance (microgram)
- (v) Conical flask
- (vi) Measuring cylinder

Procedure

Take 50 ml of well mixed sewage sample in a measuring cylinder. Have four folds of the standard filter paper and fix it on the funnel placed over a conical flask. Pour the sewage gently on the funnel and allow it to slowly filter down through the funnel shaped filter paper. Pour it intermittently so that the filtrate is only sewage containing dissolved solids and the suspended impurities are filtered out.

Transfer filtrate to a weighed evaporating dish (weight say A mg) and evaporate to dryness in the drying oven. Dry evaporated sample for 1 hr in an oven at 180°C and cool it. Weight it say as B mg, and calculate the dissolved

CALCULATIONS AND RESULT

$$\text{Total Dissolved Solids in mg/litre} = \frac{(A-B) \times 1000}{50 \text{ (volume of sample in ml)}}$$

Comments

The total dissolved solids give an idea about the organic and inorganic matter present in the sewage in dissolved form. Organic matter is volatile and can be determined by igniting the residue at higher temperature at 550 °C. Even the total dissolved solids give a fair idea about the organic matter and the anticipated treatment of the wastewater. Treatment means to satisfy the BOD. BOD can be satisfied aerobically or anerobically. Aerobic treatment is better as it produces less harmful end products but it is generally costly. So depending upon the foulness (organic solid matter) and the funds available the selection of process is done.

The total dissolved solids in the given sewage sample aremg/L which shows that.....



Drying oven

Experiment No-3

3a. Determination of Alkalinity of Water

Aim

To determine the amount of the following types of alkalinity present in the given samples:

- a. Hydroxide alkalinity
- b. Carbonate alkalinity
- c. Bicarbonate alkalinity
- d. Hydroxide–Carbonate alkalinity
- e. Carbonate–Bicarbonate alkalinity

Principle

The alkalinity of water is a measure of its capacity to neutralize acids. It is primarily due to salts of weak acids, although weak or strong bases may also contribute. Alkalinity is usually imparted by bicarbonate, carbonate and hydroxide. It is measured volumetrically by titration with 0.02 N sulphuric acid and is reported in terms of CaCO_3 equivalent. For samples whose initial pH is above 8.3, the titration is conducted in two steps. In the first step, the titration is conducted until the pH is lowered to 8.2, the point at which phenolphthalein indicator turns from pink to colourless. This value corresponds to the points for conversion of carbonate to bicarbonate ion. The second phase of titration is conducted until the pH is lowered to 4.5, corresponds to methyl orange end point, which corresponds to the equivalence points for the conversion of bicarbonate ion to carbonic acid.

Apparatus

1. Burette
2. Erlenmeyer flask
3. Pipettes



Erlenmeyer flask

Reagents

1. Carbon dioxide free distilled water.
2. Phenolphthalein indicator.
3. Methyl orange indicator.
4. 0.1 N sodium thiosulphate solution
5. 0.02 N sulphuric acid.

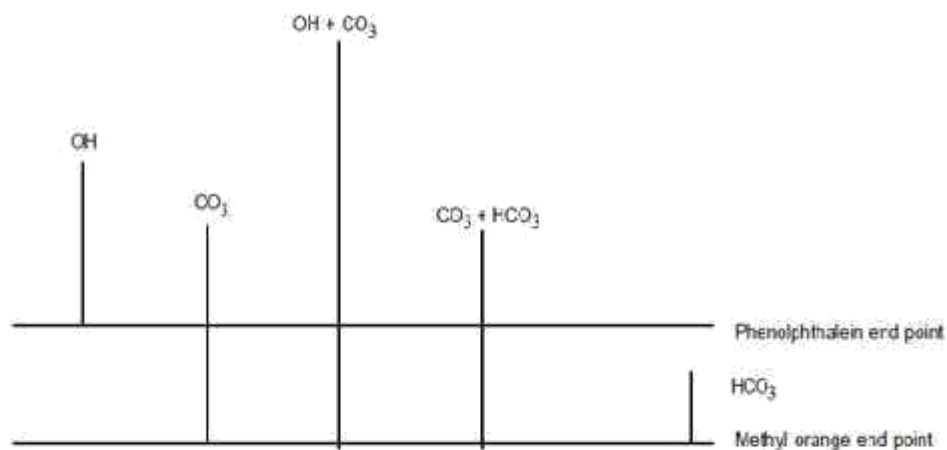
Reagents preparation:

1. **0.02 N standard sulphuric acid:** Prepare stock solution approximately 0.1 N by diluting 2.5 mL concentrated sulphuric acid to 1 litre. Dilute 200 mL of the 0.1 N stock solution to 1 litre CO₂ free distilled water. Standardise the 0.02 N acid against a 0.02 N sodium carbonate solution which has been prepared by dissolving 1.06 g anhydrous Na₂CO₃ and diluting to the mark of a 1 litre volumetric flask.
2. **Methyl orange indicator:** Dissolve 500 mg methyl orange powder in distilled water and dilute it to 1 litre. Keep the solution in dark or in an amber coloured bottle.

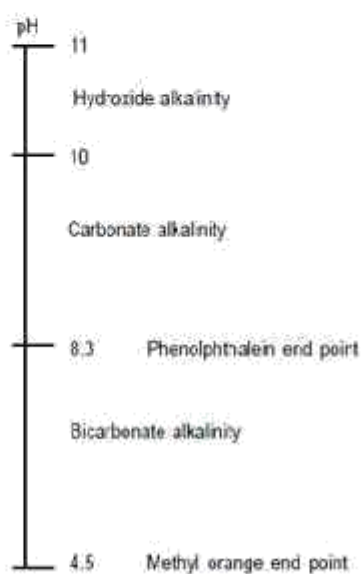
3. **Phenolphthalein indicator:** Dissolve 5 g phenolphthalein in 500mL ethylalcohol and add 500 mL distilled water. Then add 0.02 N sodium hydroxide drop-wise until a faint-pink colour appears.
4. **Sodium thiosulphate 0.1 N:** Dissolve 25 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ and dilute to 1litre.

Procedure

1. Pipette 50 mL of sample into a clean Erlenmeyer flask (V).
2. Add one drop of sodium thiosulphate solution, if residual chlorine is present.
3. Add two drops of phenolphthalein indicator; if the pH is above 8.3, colour of solution becomes pink.
4. Titrate against standard sulphuric acid in the burette, till the colour just disappears. Note down the volume (V1).
5. Then add two drops of methyl orange indicator, the colour turns yellow.
6. Again titrate against acid, until the colour turns to orange yellow. Note down the total volume (V2).



Graphical Representation of Titration of Samples Containing Various Forms of Alkalinity



Observation

0.02 N H₂SO₄ x sample (Methyl orange/phenolphthalein indicator)

Description of sample	Trial no.	Burette reading (phenolphthalein indicator)		Volume of acid used V_1	Burette reading (methyl orange indicator)		Volume of acid used V_2
		Initial	Final		Initial	Final	

Calculation

$$1. \text{ Phenolphthalein alkalinity (P) as mg/L CaCO}_3 = \frac{V_1 \times 1000}{\text{mL of sample}}$$

$$2. \text{ Total alkalinity (T) as mg/L CaCO}_3 = \frac{V_2 \times 1000}{\text{mL of sample}}$$

The types of alkalinities present in the samples are calculated using the equations given in the following table and the results are tabulated.

Result of titration	Hydroxide alkalinity as CaCO_3	Carbonate alkalinity as CaCO_3	Bicarbonate alkalinity as CaCO_3
$P = 0$	0	0	T
$P < \frac{1}{2}T$	0	2P	$T - 2P$
$P = \frac{1}{2}T$	0	2P	0
$P > \frac{1}{2}T$	$2P - T$	$2(T - P)$	0
$P = T$	T	0	0

Results :

<i>Description of sample</i>	<i>Hydroxide alkalinity as CaCO_3 in mg/L</i>	<i>Carbonate alkalinity as CaCO_3 in mg/L</i>	<i>Bicarbonate alkalinity as CaCO_3 in mg/L</i>	<i>Hydroxide carbonate alkalinity as CaCO_3 in mg/l</i>	<i>Carbonate bicarbonate alkalinity as CaCO_3 in mg/L</i>

Viva Questions:

1. What is the importance of total dissolved solids?
2. What are the apparatus used for total dissolved solids?

Experiment No-3

3b. Determination of Acidity

Aim

To determine the acidity of the given sample of water.

Principle Acidity of water is its quantitative capacity to neutralise a strong base to a designated pH. Strong mineral acids, weak acids such as carbonic and acetic and hydrolysing salts such as ferric and aluminium sulphates may contribute to the measured acidity. According to the method of determination, acidity is important because acid contributes to corrosiveness and influences certain chemical and biological processes. It is the measure of the amount of base required to neutralise a given sample to the specific pH.

Hydrogen ions present in a sample as a result of dissociation or hydrolysis of solutes are neutralised by titration with standard alkali. The acidity thus depends upon the end point pH or indicator used. Dissolved CO_2 is usually the major acidity component of unpolluted surface water. In the sample, containing only carbon dioxide-bicarbonate-carbonate, titration to pH 8.3 at 25°C corresponds to stoichiometric neutralisation of carbonic acid to carbonate. Since the colour change of phenolphthalein indicator is close to pH 8.3, this value is accepted as a standard end point for the titration of total acidity. For more complex mixture or buffered solution fixed end point of pH 3.7 and pH 8.3 are used. Thus, for standard determination of acidity of wastewater and natural water, methyl orange acidity (pH 3.7) and phenolphthalein acidity (pH 8.3) are used.

Thus, in determining the acidity of the sample the volumes of standard alkali required to bring about colour change at pH 8.3 and at pH 3.7 are determined.

Apparatus

1. Burette

2. Pipette
3. Erlenmeyer flasks
4. Indicator solutions

Reagents

1. CO₂ free water
2. Standard NaOH solution 0.02N
3. Methyl orange indicator solution
4. Phenolphthalein indicator solution
5. Sodium thiosulphate 0.1 N.
6. **NaOH solution 0.02 N:** Dissolve 4 g NaOH in 1 litre water. This gives 0.1N NaOH solution. Take 200 ml of this 0.1 N solution and make it up to 1 litre to obtain 0.02 N NaOH solution.
7. **Methyl orange indicator:** Dissolve 500 mg methyl orange powder in distilled water and dilute it to 1 litre.
8. **Phenolphthalein indicator:** Dissolve 5 g phenolphthalein disodium salt in distilled water and dilute to 1 litre.
9. **Sodium thiosulphate 0.1 N:** Dissolve 25 g Na₂S₂O₃·5H₂O and dilute to 1 litre distilled water.

Procedure

1. 25 mL of sample is pipette into Erlenmeyer flask.
2. If free residual chlorine is present, 0.05 mL (1 drop) of 0.1 N thiosulphate solution is added.
3. 2 drops of methyl orange indicator is added.
4. These contents are titrated against 0.02 N hydroxide solution. The end point is noted when colour change from orange red to yellow.
5. Then two drops of phenolphthalein indicator is added and titration continued till a pink colour just develops. The volumes of the titrant used are noted down.

Observation

0.02 N NaOH × Sample (Methyl orange/phenolphthalein indicator)

Description of sample	Trial no.	Burette reading		Volume of NaOH used A
		Initial	Final	

Calculation

Acidity in mg/L as CaCO_3 =

where,

A = mL of NaOH titrant B = normality of NaOH V = mL of the sample.

Results

Sample no.	Acidity in mg/L as CaCO_3

Viva questions:

1. Define the significance of the experiment acidity?
2. What are the apparatus used in finding out the experiment?

Experiment No-4

Determination of Chloride in Water

Aim

To determine the amount of chloride (in the form of Cl^-) present in the given water sample by Mohr's method.

Principle

If water containing chlorides is titrated with silver nitrate solution, chlorides are precipitated as white silver chloride. Potassium chromate is used as indicator, which supplies chromate ions. As the concentration of chloride ions approaches extinction, silver ion concentration increases to a level at which reddish brown precipitate of silver chromate is formed indicating the end point.

Apparatus

1. Burette
2. Pipettes
3. Erlenmeyer flasks
4. Measuring cylinder

Reagents;

1. Chloride free distilled water.
2. Standard silver nitrate solution (0.0141N)
3. Potassium chromate indicator.
4. Acid or alkali for adjusting pH.
5. **Potassium chromate indicator:** Dissolve 50 g potassium chromate($\text{K}_2\text{Cr}_2\text{O}_4$) in a little distilled water. Add silver nitrate solution until a definite red precipitate is formed. Let stand for 12 hours, filter and dilute the filtrate to 1 litre with distilled

water.

6. **Standard silver nitrate solution 0.0141 N:** Dissolve 2.395 g AgNO_3 in distilled water and dilute to 1 litre. Standardise against 0.0141 N NaCl. Store in a brown bottle; 1 mL = 500 $\mu\text{g Cl}_2$.
7. **Standard sodium chloride 0.0141N:** Dissolve 824.1 mg NaCl (dried at 140°C) in chloride free water and dilute to 1 litre. 1 mL = 500 $\mu\text{g Cl}_2$.
8. **Aluminium hydroxide suspension:** Dissolve 125 g aluminium potassium sulphate in 1 litre water. Warm to 60°C and add 55 mL concentrated NH_4OH slowly with stirring. Let stand for 1 hour, transfer the mixture to a large bottle. When freshly prepared the suspension occupies a volume of approximately 1 litre.

Procedure

1. Take 50 mL of sample (V) and dilute to 100 mL.
2. If the sample is coloured add 3 mL of aluminium hydroxide, shake well; allow to settle, filter, wash and collect filtrate.
3. Sample is brought to pH 7-8 by adding acid or alkali as required.
4. Add 1 mL of indicator (Potassium chromate).
5. Titrate the solution against standard silver nitrate solution until a reddish brown precipitate is obtained.
Note down the volume (V_1).
6. Repeat the procedure for blank and note down the volume (V_2).

Observation

Water sample vs Silver nitrate (0.0141 N) (Potassium chromate indicator)						
Sample no.	Trial no.	Volume of sample (mL)	Burette reading		Volume of silver nitrate (mL)	Chloride mg/L
			Initial	Final		
1	1					
	2					
	3					
2	1					
	2					
	3					
3	1					
	2					
	3					
Distilled Water	1					
	2					
	3					

$$V =$$

$$V_1 =$$

$$V_2 =$$

$$N =$$

$$\text{Chloride in mg/L} = \frac{(V_1 - V_2) \times N \times 35.46 \times 1000}{V}$$

$$= \frac{(V_1 - V_2) \times 500}{V} = \dots\dots\dots \text{mg/L}$$

Result :

Viva Questions:

1. What are the significance of chlorine demand?
2. Why chlorine is important in water used for environmental tests?

Experiment No-5

Determination of Iron

Aim

To determine the quantity of iron present in the given sample of water.

Principle

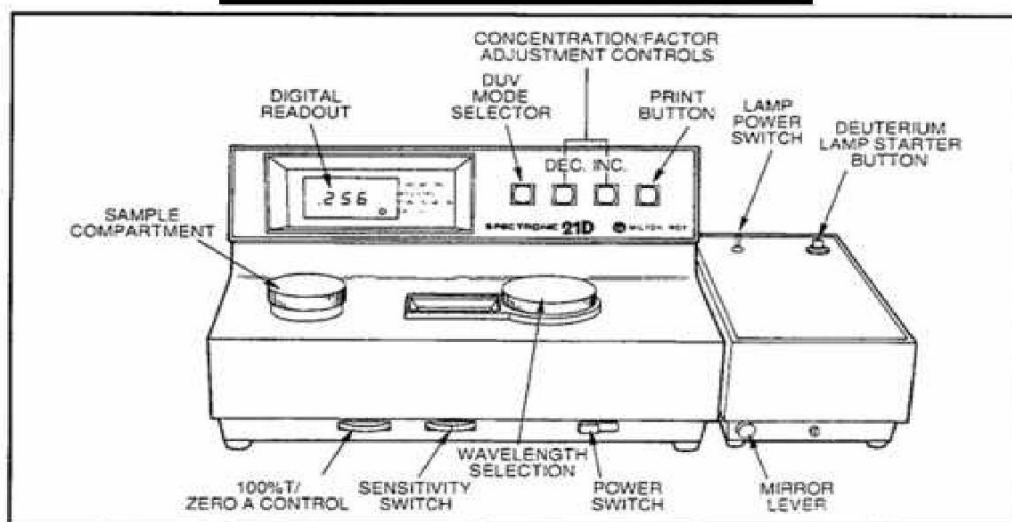
Iron is usually present in natural water and is not objectionable, if concentration is less than 0.3 ppm. It may be in true solution in colloidal state that may be peptized by organic matter, in the inorganic and organic iron complexes, or in relatively coarse suspended particles. It may be either ferrous or ferric, suspended or filterable. Iron exists in soils and minerals mainly as insoluble ferric oxide and iron sulphide (pyrite). It occurs in some areas, also as ferrous carbonate (siderite), which is very slightly soluble.

The phenanthroline method is the preferred standard procedure for the measurement of iron in water except when phosphate or heavy metal interferences are present. The method depends upon the fact that 1, 10-phenanthroline combine with Fe^{++} to form an orange-red complex. Its colour conforms to Beer's law and is readily measured by visual or photometric comparison. Small concentration of iron can be most satisfactorily determined by colorimetric analysis. It is also based on Beer's law. By measuring the intensities of transmitted and incident light through a coloured solution and knowing its optical density or transmission, we can prepare a calibration curve and subsequent concentration can be read.

Phenanthroline Method

Apparatus

1. Colorimetric equipment; one of the following is required:
 - (a) Spectrophotometer, for use at 510 nm, providing a light path of 1 cm or longer.
 - (b) Nessler tubes, matched, 100 mL, tall form.
2. Glassware like conical flasks, pipettes and glass beads.



Reagents

1. Hydrochloric acid
2. Hydroxylamine solution
3. Ammonium acetate buffer solution

4. Sodium acetate solution
5. Phenanthroline solution
6. Stock iron solution
7. Standard iron solution (1 mL = 10 µg Fe)

Procedure

1. Pipette 10, 20, 30 and 50 mL. Standard iron solution into 100 mL conical flasks.
2. Add 1 mL hydroxylamine solution and 1 mL sodium acetate solution to each flask.
3. Dilute each to about 75 mL with distilled water.
4. Add 10 mL phenanthroline solution to each flask.
5. Make up the contents of each flask exactly to 100mL by adding distilled water and left stand for 10 minutes.
6. Take 50 mL distilled water in another conical flask.
7. Repeat steps 2 to 5 described above.
8. Measure the absorbance of each solution in a spectrophotometer at 508 nm against the reference blank prepared by treating distilled water as described in steps 6 and 7. Prepare a calibration graph taking meter reading on y-axis and concentration of iron on x-axis.
9. For visual comparison, pour the solution in 100 mL tall form Nessler tubes and keep them in a stand.
10. Mix the sample thoroughly and measure 50 mL into a conical flask.
11. Add 2 mL conc. hydrochloric acid (HCl) and 1mL hydroxylamine solution. Add a few glass beads and heat to boiling. To ensure dissolution of all the iron, continue boiling until the volume is reduced to 15 to 20 mL.
12. Cool the flask to room temperature and transfer the solution to a 100 mL Nessler tube.
13. Add 10 mL ammonium acetate buffer solution and 2 mL phenanthroline solution and dilute to the 100 mL mark with distilled water.
14. Mix thoroughly and allow at least 10 to 15 minutes for maximum colour development.

15. Measure the absorbance of the solution in a 1cm cell in a spectrophotometer at 508 nm.
16. Read off the conc. of iron (mg Fe) from the calibration graph for the corresponding meter reading.
17. For visual comparison, match the colour of the sample with that of the standard prepared in steps 1 to 7 above.
18. The matching colour standard will give the concentration of iron in the sample ($\mu\text{g Fe}$).

Observation

<i>Standard iron solution in mL</i>	<i>Iron content in μg</i>	<i>Absorbance</i>

<i>Sample no.</i>	<i>Absorbance</i>	<i>Iron content from graph in μg</i>	<i>Iron as Fe in mg/L</i>

Sample Calculation

iron (Fe) in mg/L = $\mu\text{g Fe/mL}$ of sample =
 mg/L

Result:

viva questions:

1. what is the significance of the experiment iron?
2. what are the apparatus used for finding out iron tests

Experiment No-6

Determination of Dissolved Oxygen in Water

Aim

The aim of the experiment is to determine the quantity of dissolved oxygen present in the given sample(s) by using modified Winkler's (Azide modification) method.

Principle

Dissolved Oxygen (D.O.) levels in natural and wastewaters are dependent on the physical, chemical and biochemical activities prevailing in the water body. The analysis of D.O. is a key test in water pollution control activities and waste treatment process control.

Improved by various techniques and equipment and aided by instrumentation, the Winkler (or iodometric) test remains the most precise and reliable titrimetric procedure for D.O. analysis. The test is based on the addition of divalent manganese solution, followed by strong alkali to the water sample in a glass-stoppered bottle. D.O. present in the sample rapidly oxidises in equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states. In the presence of iodide ions and upon acidification, the oxidised manganese reverts to the divalent state, with the liberation of iodine equivalent to the original D.O. content in the sample. The iodine is then titrated with a standard solution of thiosulphate.

Apparatus

1. 300 mL capacity bottle with stopper
2. Burette
3. Pipettes, etc.

Reagents

1. Manganoussulphate solution ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$)
2. Alkali-iodide azide reagent
3. Conc. sulphuric acid (36 N)
4. starch indicator
5. Standard sodium thiosulphate solution (0.025N)
6. Standard potassium dichromate solution (0.025N)

1. **Manganoussulphate solution:** Dissolve 480 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 400 g $\text{MnSO}_2 \cdot 2\text{H}_2\text{O}$ or 364 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in distilled water, filter and dilute to 1 litre.
2. **Alkali-iodide-azide reagent:** Dissolve 500 g NaOH or 700 g KOH and 135 g NaI or 150 g KI in distilled water and dilute to 1 litre. Add 10 g sodium azide (NaN_3) dissolved in 40 mL distilled water. The reagent should not give colour with starch when diluted and acidified.
3. **Sulphuric acid concentrated:** 1mL is equivalent to about 3 mL alkali-iodide-azide reagent.
4. **Standard sodium thiosulphate 0.025 N:** Dissolve 6.205 g sodiumthiosulphate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) in freshly boiled and cooled distilled water and dilute to 1 litre. Preserve by adding 5 mL chloroform or 0.4 g NaOH/L or 4 g borax and 5 10 mg HgI_2 /L. Standardise this with 0.025 N potassium dichromate solution which is prepared by dissolving 1.226 g potassium dichromate in distilled water and diluted to 1 litre.
5. **Standard potassium dichromate solution 0.025 N:** A solution of potassium dichromate equivalent to 0.025 N sodium thiosulphate contains 1.226 g/L $\text{K}_2\text{Cr}_2\text{O}_7$. Dry $\text{K}_2\text{Cr}_2\text{O}_7$ at 103°C for 2 hrs before making the solution.
6. **Standardisation of 0.025 N sodium thiosulphatesolution:** Dissolve approximately 2 g KI in an Erlenmeyer flask with 100 to 150 mL distilled water. Add 10 mL of H_2SO_4 , followed by exactly 20 mL, 0.1 N potassium dichromate solution. Place in the dark for 5 minutes, dilute to approximately 400 mL and titrate with 0.025 N sodium thiosulphate solution, adding starch towards the end of titration. Exactly 20 ml 0.025 N thiosulphate will be consumed at the end of the titration. Otherwise, the

thiosulphate solution should be suitably corrected.

7. **Starch Indicator:** Add cold water suspension of 5 g soluble starch to approximately 800 mL boiling water with stirring. Dilute to 1 litre, allow to boil for a few minutes and let settle overnight. Use supernatant liquor.

Preserve with 1.25 g salicylic acid/1 litre or by the addition of a few drops of toluene.

Procedure

1. Add 2 mL of manganous sulphate solution and 2 mL of alkali-iodide azide reagent to the 300 mL sample taken in the bottle, well below the surface of the liquid.

(The pipette should be dipped inside the sample while adding the above two reagents.)

2. Stopper with care to exclude air bubbles and mix by inverting the bottle at least 15 times.
3. When the precipitate settles, leaving a clear supernatant above the manganese hydroxide floc, shake again.
4. After 2 minutes of settling, carefully remove the stopper, immediately add 3 mL concentrated sulphuric acid by allowing the acid to run down the neck of the bottle.
5. Restopper and mix by gentle inversion until dissolution is complete.
6. Measure out 203 mL of the solution from the bottle to an Erlenmeyer flask. As 2 mL each of manganese sulphate and azide reagent have been added, the proportionate quantity of yellow solution corresponds to 200 mL of sample is

$$= \frac{200 \times 300}{300 - 4} = 203 \text{ mL}$$

7. Titrate with 0.025 N sodium thiosulphate solution to a pale straw colour.
8. Add 1–2 mL starch solution and continue the titration to the first disappearance of the blue colour and note down the volume of sodium thiosulphate solution added (V), which gives directly the D.O. in mg/L.

Observation

Sample x Standard sodium thiosulphate solution (0.025N) (Starch indicator)

Description of sample	Trial no.	Volume of sample (mL)	Burette reading		Volume of titrant mL	D.O. in mg/L
			Initial	Final		
Sample I						
Sample II						
Sample III						

Result

Description of sample	D.O. mg/L
Sample I	
Sample II	
Sample III	

Viva Questions:

1. What is the significance of the experiment dissolved oxygen?
2. Define dissolved oxygen of water.

Experiment No-7

Determine the Nitrate Nitrogen

AIM: To determine the nitrate nitrogen of the given sample of water.

Principle

The reaction with the nitrate and brucine produces yellow colour that can be used for the colorimetric estimation of nitrate. The intensity of colour is measured at 410 nm. The method is recommended only for concentration of 0.1– 2.0 mg/L NO_3^- -N. All strong oxidising and reducing agent interfere. Sodium arsenite is used to eliminate interference by residual chlorine; sulphanilic acid eliminates the interferences by NO_2^- -N and chloride interference is masked by addition of excess NaCl. High concentration of organic matter also may interfere in the determination.

Apparatus

1. Spectrophotometer
2. Water bath
3. Reaction tubes
4. Cool water bath

Reagents

1. Stock nitrate solution
2. Standard nitrate solution
3. Sodium arsenite solution
4. Brucine-sulphanilic acid solution
5. Sulphuric acid solution
6. Sodium chloride solution



Water Bath



Spectrometer

Procedure

1. Nitrate standards are prepared in the range 0.1–1.0 mg/LN diluting 1.00, 2.00, 4.00, 7.00 and 10.0 mL standard nitrate solution to 10 mL with distilled water.
2. If residual chlorine is present 1 drop of sodium arsenite solution is added for each 0.1 mg Cl_2 and mixed.
3. Set up a series of reaction tubes in test tube stand. Add 10 mL sample or a portion diluted to 10 mL to the reaction tubes.
4. Place the stand in a cool water bath and add 2 mL NaCl solution and mix well.
5. Add 10 mL H_2SO_4 solution and again mix well and allow cooling.

6. The stand is then placed in a cool water bath and add 0.5 ml brucine-sulphanilic acid reagent. Swirl the tubes and mix well and place the tubes in boiling water bath at temperature 95°C.
7. After 20 minutes, remove the samples and immerse in cool water bath.
8. The sample are then poured into the dry tubes of spectrophotometer and read the standards and sample against the reagent blank at 410 nm.
9. Prepare a standard curve for absorbance value of standards (minus the blank) against the concentration of NO₃N.
10. Read the concentration of NO₃N in the sample from the known value of absorbance.

Calculation

$$\text{Nitrate N in mg/L} = \frac{\mu\text{g NO}_3^- - \text{N}}{\text{mL of sample}}$$

$$\text{NO}_3 \text{ in mg/L} = \text{mg/L nitrate N} \times 4.43.$$

Observation

The observation are presented in Tables A and B respectively.

Table A: Observation for calibration

Stock nitrate solution in mL	Nitrate	Absorbance

Table B:

Sample no.	Absorbance	Nitrate nitrogen in μg from graph	Nitrate nitrogen in mg

Results

<i>Sample no. or description</i>	<i>Nitrate nitrogen in mg/L</i>

Viva Questions:

1. What is the significance of the experiment?
2. Define nitrogen oxygen.

Experiment No-8

Determination of Optimum Dose of Coagulant

Aim :

To determine the optimum coagulant dosage for clarifying the given sample of water by using alum as the coagulant and performing the jar test experiment.

Principle

Coagulants are used in water treatment plants

- (i) to remove natural suspended and colloidal matter,
- (ii) to remove material which do not settle in plain sedimentation, and
- (iii) to assist in filtration.

Alum [$\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$] is the most widely used coagulant. When alum solution is added to water, the molecules dissociate to yield SO_4^{2-} and Al^{3+} . The +ve species combine with negatively charged colloidal to neutralize part of the charge on the colloidal particle. Thus, agglomeration takes place. Coagulation is a quite complex phenomenon and the coagulant should be distributed uniformly throughout the solution. A flash mix accomplishes this.

Jar test is simple device used to determine this optimum coagulant dose required. The jar test, device consists of a number of stirrers (4 to 6) provided with paddles. The paddles can be rotated with varying speed with the help of a motor and regulator. Samples will be taken in jars or beakers and varying dose of coagulant will be added simultaneously to all the jars. The paddles will be rotated at 100 rpm for 1 minute and at 40 rpm for 20 to 30 minutes, corresponding to the flash mixing and slow mixing in the flocculator of the treatment plant. After 30 minutes settling, supernatant will be taken carefully from all the jars to measure turbidity. The dose, which gives the least turbidity, is taken as the optimum coagulant dose.

Apparatus :

1. Jar Test Apparatus
2. Glass Beakers
3. Pipette
4. Nephelometer
5. pH meter

Reagents

1. Alum solution (1mL containing 10 mg of alum)
2. Lime
3. Acid/alkali

Procedure

1. Take 1-litre beakers and fill them with sample up to the mark.
2. Keep each beaker below each paddle and lower the paddles, such that each one is about 1cm above the bottom.
3. Find the pH of the sample and adjust it to 6 to 8.5.
4. Pipette 1, 2, 3, 4, 5, 6 mL of the alum solution into the test samples.
5. Immediately run the paddles at 100 rpm for 1 minute.
6. Reduce the speed to 30–40 rpm and run at this rate for 30 minutes.
7. Stop the machine, lift out the paddles and allow to settle for 30 minutes.
8. Find the residual turbidity of the supernatant using nephelometer.
9. Plot a graph with alum dosage along x-axis and turbidity along y-axis.
10. The dosage of alum, which represents least turbidity, gives Optimum Coagulant Dosage (O.C.D.).
11. Repeat steps 1–10 with higher dose of alum, if necessary.

Observation:

<i>Trial No.</i>	<i>Alum Dosage in mg/L</i>	<i>Turbidity in NTU</i>

Results:

Optimum coagulant dosage =

Viva Questions:

1. What is optimum dosage of water?
2. What is significance of optimum dosage of water?

Experiment No-9

Determination of Chlorine Demand

Chlorine Dose, Demand, and Residual

Most water treatment plants are required to **disinfect** the water, a process used to kill harmful bacteria. The most frequently used method of disinfection is the addition of chlorine. Here, we will briefly introduce three terms used during chlorination - chlorine dose, chlorine demand, and chlorine residual. These three characteristics are related to each other using the following equation:

$$(\text{Chlorine demand}) = (\text{Chlorine dose}) - (\text{Chlorine residual})$$

The amount of chlorine added to the water is known as the **chlorine dose**. This is a measured quantity chosen by the operator and introduced into the water using a chlorinator or hypochlorinator.

As the chlorine reacts with bacteria and chemicals in the water, some of the chlorine is used up. The amount of chlorine used up by reacting with substances in the water is known as the **chlorine demand**. If nothing reacts with the chlorine (as would be the case in distilled water), then the chlorine demand is zero. However, in most cases the operator should count on some of the chlorine dose being used up when it reacts with substances in the water.

The amount of chlorine remaining in the water after some of the chlorine reacts with substances in the water is known as the **chlorine residual**. This lab introduces a test which can be used to calculate the chlorine residual. The chlorine residual is the most important of these three values - dose, demand, and residual - because it represents the actual amount of chlorine remaining in the water to act as a disinfect

The test for chlorine residual is performed frequently at most water treatment plants. Since regulations require a certain level of chlorine in water at the far ends of the distribution system, operators should be sure to test the chlorine residual in the distribution system as well as in the clear well.

Introduction to Testing Procedures

The DPD Colorimetric Method introduced in this lab is one of several procedures which can be used to test for chlorine residual. This method requires compensation for color and turbidity and can detect chlorine concentrations only as low as 10 ug as Cl₂/L. *Standard Methods* introduces several other procedures and explains which procedures are most effective under a variety of circumstances. In every case, remember that chlorine is a relatively volatile substance and that samples should be tested as soon as possible after the water is collected.

The chemistry involved in the DPD Colorimetric Method is relatively simple. The buffer lowers the pH of the sample to 4 or less. In this pH range, chlorine in the water is able to react with the added potassium iodide, replacing the iodine which is released into the solution as shown below:



When free iodine becomes present in the water, the indicator makes the solution change to a red color, with the intensity of the color equivalent to the amount of chlorine found in the solution.

Equipment

This procedure requires a piece of colorimetric equipment, some glassware, and Titration equipment. The colorimetric equipment must be one of the following:

1. Spectrophotometer, for use at a wavelength of 515 nm and providing a light path of 1 cm or longer.
2. Filter photometer, equipped with a filter having maximum transmission in

the wavelength range of 490 to 530 nm and providing a light path of 1 cm or longer.

Reagents

1. Standard potassium permanganate solutions
2. Phosphate buffer solution
3. N,N-Diethyl-p-phenylenediamine (DPD) indicator solution
4. Standard ferrous ammonium sulfate (FAS) titrant
5. Potassium iodide (KI) crystals
6. Chlorine-demand-free water

(For information on how to prepare these reagents, see p. 4-62 in *Standard Methods*.)

Procedure

1. Calibrate the photometric equipment using the following procedure. Note that this procedure uses potassium permanganate solutions. You can use chlorine solutions to calibrate the equipment by following the procedure in *Standard Methods* on pages 4-63 to 4-64.

1. Set 100%T on the spectrophotometer or filter photometer using a distilled water blank, in accordance with manufacturer's instructions. (Prepare the distilled water blank in the same manner as you prepare the sample for testing.)
2. Prepare a series of potassium permanganate standards covering the equivalent chlorine range of 0.05 to 4 mg/L. (The procedure for producing the standards is explained in *Standard Methods* on page 4-64.)
3. Label empty flasks for each standard. Place 5 mL of phosphate buffer and 5 mL of DPD indicator reagent in each labelled flask.
4. Add 100 mL of each prepared potassium permanganate standard solution to the appropriate flask and mix thoroughly.

5. Fill a photometer or colorimeter cell with the solution in each flask and read each standard at a wavelength of 515 nm.
6. In the data section, plot a standard curve of mg/L equivalent chlorine versus %T.
7. Return the cell contents to the appropriate flask and titrate with FAS titrant as a check on any absorption of permanganate by distilled water.

2. Measure the chlorine content of the sample.

- a) Pipette 0.5 mL of phosphate buffer solution into an empty test tube.
- b) Add 0.5 mL of DPD indicator solution to the test tube.
- c) Add 10 mL of sample water and read the color immediately. Use the standard curve to determine the amount of chlorine in the sample. Record this in the Data section as Reading A.
- d) Continue by adding one very small crystal of KI (about 0.1 mg) to the test tube and mixing. Read the color immediately. Use the standard curve to determine the amount of chlorine in the sample. Record this in the Data section as Reading B.
- e) Continue by adding several crystals of KI (about 0.1 g) to the test tube and mixing. Let the solution stand for about two minutes to allow color to develop, then read the color. Use the standard curve to determine the amount of chlorine in the sample. Record this in the Data section as Reading C.
- f) Place a very small crystal of KI (about 0.1 mg) in a clean test tube. Add 10 mL of the sample and mix. In a separate tube, add 0.5 mL of the phosphate buffer solution and 0.5 mL of the DPD indicator solution and mix. Add the contents of the second tube to the first tube and mix. Read the color immediately. Use the standard curve to determine the amount of chlorine in the sample. Record this in the Data section as Reading N.

3. Calculate the amount of each type of chlorine using the calculation methods listed in Table 2 in the Data section. For example, let's consider our calculations

adings were $A = 1.0 \text{ mg/L}$, $B = 1.3 \text{ mg/L}$, $C = 2.7 \text{ mg/L}$, and $N = 1.9 \text{ mg/L}$.

First, the amount of free chlorine was shown by Reading A to be 1.0 mg/L .

The amount of monochloramine is calculated as:

$$B - A = 1.3 \text{ mg/L} - 1.0 \text{ mg/L} = 0.3 \text{ mg/L}$$

So the concentration of monochloramine is 0.3 mg/L .

Since N is more than 0, the dichloramine concentration is calculated as: $C - N =$

$$2.7 \text{ mg/L} - 1.9 \text{ mg/L} = 0.8 \text{ mg/L}$$

So the concentration of dichloramine is 0.8 mg/L .

Finally, since N is more than 0 and there are monochloramines present, the amount of trichloramine is calculated as follows:

$$2(N - B) = 2(1.9 \text{ mg/L} - 1.3 \text{ mg/L}) = 1.2 \text{ mg/L}$$

So the concentration of trichloramine is 1.2 mg/L .

Data:

Standard Curve

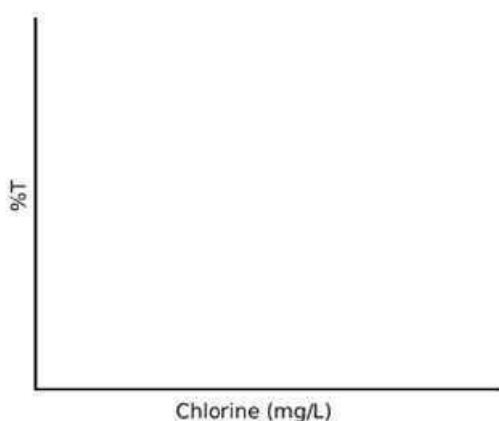


Table 1

Reading	%T	mg/L chlorine
A		
B		
C		
N		

Table 2

Type of Chlorine	Calculation Method	mg/L
Free Chlorine	A	
Monochloramine	B - A	
Dichloramine	If N=0, then C - B; If N>0, then C - N	
Trichloramine	If N=0, then 0 mg/L; If N>0 and (B - A) = 0, then 2(N - A) If N>0 and (B - A)>0, then 2(N - B)	

Result:

Viva Questions:

1. Define chlorine demand?
2. What is the significance of the experiment chlorine demand?

Experiment No-10

Determination of Total Phosphorus

Determination of Total Phosphorus

Persulfate digestion followed by Ascorbic acid Method

Reference: Standard Methods, 18th edition, Procedure 4500-PB (5) & 4500 –PE

Apparatus:

1. Hot plate (An autoclave may be used in place of a hot plate.)
2. Bausch & Lomb Spectronic 100 spectrophotometer or equivalent
3. Cuvettes. If multiple cuvettes are used, they should be a matched set.

Note: Use only glassware designated for phosphorous analyses. Wash glassware with a phosphate-free detergent and 1:1 hydrochloric acid after each use.

Reagents:

1. **Phenolphthalein** indicator. Make phenolphthalein indicator solution by dissolving 0.1 g phenolphthalein in 50 mL of ethyl alcohol. Add 50 mL distilled water. Or purchase commercially prepared product.
2. **Sulfuric acid solution (30%)**: Slowly add 300 mL conc. H₂SO₄ to approximately 600 mL distilled water that has been placed in a beaker or flask immersed in cold water. DO NOT ATTEMPT TO MIX THIS SOLUTION IN THE SULFURIC ACID BOTTLE. Allow to thoroughly cool and dilute to 1 L.
3. Ammonium persulfate: (NH₄)₂S₂O₈, crystalline
4. Sodium hydroxide: (NaOH), 1N.
5. **Stock Phosphate solution, 50 mg/L**: Dissolve 219.5 mg (0.2195g) potassium dihydrogen phosphate (KH₂PO₄) in reagent water. Dilute to 1 L. 1 mL = 50.0 ug (0.050 mg) PO₄³⁻-P [as phosphorous].

NOTE: It is recommended that KH₂PO₄ be purchased from two different suppliers. The material from one source should be used to prepare calibration stock standards, while

the other source is used to prepare stock standards used to prepare spike samples. If the same solution that is used to prepare calibration standards is also used to prepare spiked samples, errors made in the preparation of the stock standard cannot be easily identified. (Commercially prepared standard solutions may be purchased with certified concentrations).

6. **Standard Phosphate solution, 2.5 mg/L:** Pipette 25.0 mL of the stock solution into a 500 mL volumetric flask.
7. **Potassium antimonyl tartrate reagent:** Dissolve 1.3715 g $K(SbO)C_4H_4O_7 \cdot \frac{1}{2} H_2O$ in 400 mL reagent water in a 500 mL volumetric flask. Dilute to volume. Store in a glass-stopped bottle.
8. **Ammonium molybdate reagent:** Dissolve 20 g $(NH_4)_6Mo_7O_{24}$ in 500 mL reagent water in a glass-stoppered bottle.
9. **Ascorbic acid, 0.01M:** Dissolve 1.76 g ascorbic acid in 100 mL water. Store at 4°C. Discard after one week.
10. **Sulfuric acid, 5N:** Slowly add 70 mL conc. H_2SO_4 to approximately 500 mL distilled water that has been placed in a beaker or flask immersed in cold water. DO NOT ATTEMPT TO MIX THIS SOLUTION IN THE SULFURIC ACID BOTTLE. Allow to thoroughly cool.
11. **Combined reagent (Color Reagent):** For 100 mL combined reagent mix in this order 50 mL 5N sulfuric acid, 5 mL potassium antimonyl tartrate reagent, 15 mL ammonium molybdate reagent and 30 mL ascorbic acid. Let all reagents reach room temperature before combining. Mix the solutions well after each reagent is added. If the solution turns cloudy after mixing, let stand until clear. **This reagent must be used within four hours of preparation.**
12. **Color Blank Reagent:** *Prepare only if necessary*—see Sections 4 and 5. For 100 mL combined reagent mix in this order: 35 mL reagent water; 50 mL 5N sulfuric acid; and 15

mL ammonium molybdate reagent. Let all the reagents reach room temperature before combining. Mix the solutions well after each reagent is added. If the solution turns cloudy after mixing, let stand until clear.

This reagent must be used within four hours of preparation.

Calibration:

1. **Preparation of Standard Curve** *Make a new standard curve every three months or when reagents are replaced or whenever a check standard is not within 10% of true value.* If a full set of calibration standards is not prepared on each day samples are digested, then at least one known standard prepared from the stocks used to prepare the calibration standards, must be made. If the result obtained for this known standard is not within 10% of the “true”, or prepared, concentration, then a full calibration is required and samples must be re-digested.
 - a. Prepare at least three standards plus a blank at concentrations that bracket the concentration of the sample measured. This analysis has been demonstrated to be substantially non-linear beyond 1.0 mg/L. Consequently, although some newer spectrophotometers are able to extend the linear range, you should limit your calibration to an upper end of 1.0 mg/L.
 - b. Digest and test calibration standards in the same manner as the samples. Since the EPA is no longer requiring that calibration standards be digested, undigested standards are allowed provided that a mid-point known standard is prepared and digested with each set of samples processed. If the recovery of this digested standard is not within 90% to 110%, there is indication that the digestion process significantly impacts results, and calibration standards should be digested as well. Plot absorbance vs mg/L phosphate in standard to give a straight line.

Put flasks on a hot plate. *Be careful when transporting beakers! To avoid contaminating samples with phosphorus, you should be wearing laboratory gloves. Do not contact the inside of the vessel.* Boil slowly for 30 to 40 minutes or until the volume in the beaker or flask is reduced to approximately 10 mL. (**Do not allow to go to dryness.** If samples boil dry, you must discard that sample and start over with fresh glassware.)

- h. Remove the flask from the hot plate. Cool to room temperature.
- i. Add distilled water to flask until the volume is approximately 30 mL.
- j. Add one drop phenolphthalein solution.
- k. Add sodium hydroxide solution with a dropper one drop at a time until a faint (light) pink color appears. Do not add excess NaOH.
- l. Transfer the sample into a 100 mL volumetric flask; dilute to volume with reagent water.

3. Color Development

- a. Pipet 50 mL digested sample into an Erlenmeyer flask. If you know or suspect the sample to contain appreciable concentration of phosphorus, use an aliquot of sample that has been diluted to 50 mL with reagent water (e.g., if your expected sample concentration is 3 mg/L, you may wish to dilute 10 mL of digested sample to 50 mL with reagent water). Be sure to account for any such dilution when calculating sample results. Note: By “coloring”, at most, 50 mL of the digested sample, you will have sufficient volume remaining to prepare a dilution if the sample response exceeds your calibration range.
- b. Add 8.0 mL combined reagent to the 50 mL sample. Mix thoroughly.
- c. Allow the color to develop for **at least 10 minutes but no longer than 30 minutes.**

- d. Set the absorbance to zero using a digested reagent water blank. If any appreciable blue color is observed in this calibration blank, it should be noted, and corrective action should be initiated to identify the source of contamination.

NOTE: This procedure assumes that calibration standards are digested. If they are not digested, zero the spectrophotometer each day of analysis with an undigested (like the standards) reagent water blank to which color reagent has been added.

- e. Wipe the outside of each cuvette with a Kimwipe or soft tissue before inserting into the spectrophotometer. Use the same cuvette for all blanks, standards, and samples. Different cuvettes may somehow be differentially dirty or scratched leading to differences in baseline. This leads to bias in the analytical data, or may affect the ability to meet quality control limits. If multiply cuvettes are used, they should be a matched set.
- f. Rinse the cuvette between samples using the next sample to be tested.
- g. The spectrophotometer should be set at 880 nm.
- h. Read and RECORD the absorbance.

4. Calculate phosphorus concentration in sample as follows:

$$\text{mg/L total P} = \frac{\text{mg P (from curve)}}{\text{L}} \times \frac{\text{V mL} \times \text{1 L}}{1000 \text{ mL}} \times \frac{\text{FV mL}}{\text{CV mL}} \times \frac{\text{1 L}}{\text{SV mL}} \times \frac{1000 \text{ mL}}{\text{L}}$$

Where

V = volume (mL) of sample + reagent water that was colored [typically 50]

FV = final volume (mL) after digestion [typically 100]

CV = volume (mL) of sample that was colored [typically 50]

If you used 10 mL diluted to 50 with reagent water, A=10

SV = original volume of sample that was digested [typically 50]

By canceling out units, this formula can be simplified to:

$$\text{mg/L total P} = \frac{\text{mg P (from curve)}}{\text{CV} \times \text{SV}} \times \frac{\text{V} \times \text{FV}}{\text{L}} \times \text{L}$$

NOTE: If you digest 50 mL of sample, dilute to a final volume of 100 mL. take 50 mLs of the digested sample and color it, the equation simplifies to:

$$\text{mg/L total P} = \frac{\text{mg P (from curve)}}{\text{L}} \times 2$$

5. The use of “color” blanks (if the sample has appreciable color following digestion)

Some plants analyze samples that seasonally develop color due to algae or other things. This type of color in a sample will register background absorbance on the phosphorus analysis and therefore must be subtracted from the true sample signal. This requires determining the absorbance of a “color blank”.

A second aliquot of the digested sample (without the addition of combined reagent solution) should be read, recording the absorbance. This value should be subtracted from the absorbance obtained from the aliquot of the same digested sample to which combined reagent has been added.

Follow the procedure in steps 3 a and 3 b. It is important to use precisely the same amount of digested sample for “color blank” as used for the actual sample analysis.

Sample Calibration and Limit of Detection (LOD) Data from a typical WWTP: Calibration Data

Concentration	Absorbance
0	0
0.25	0.058
0.5	0.130
1.0	0.291

Slope= 3.38212347

Intercept= 0.03249071

Correlation coefficient= 0.99765256

Concentration= $\frac{\text{Absorbance} - \text{Intercept}}{\text{Slope}}$

LOD Determination

Total Phosphorus

Spike level= 0.1 mg/L

Rep.1 0.11

Rep.2 0.12

Rep.3 0.12

Rep.4 0.12

Rep.5 0.12

Rep.6 0.11

Rep.7 0.12

# replicates	t-value
7	3.143
8	2.998
9	2.896
10	2.821

mean 0.11714

st. dev 0.00488

t-value 3.143= from table based
on # replicates

MDL 0.01534= t-value x std
deviation

LOD 0.01534= roughly= to MDL

LOQ 0.05112= 10/3x the LOD

The 5-point check

1. Is the MDL no lower than 10% of the spike level? yes
2. Is the spike level greater than the calculated MDL? yes
3. Is the MDL below any relevant permit limit?
(if there is one) Permit limit? N/A
4. Is the signal-to-noise ratio (S/N) between 2.5 and 10?
CHECK
S/N = Mean/std dev.
S/N is fairly high that suggests a need to spike at a lower concentration
5. Is mean recovery within reasonably expected limits?
Yes Mean recovery= mean/spike level x 100 117.14%

Viva Questions:

1. Define the significance of total phosphorous?
2. How total phosphorous chemical is used in environmental engineering lab?

Experiment No-11

Determination of B.O.D.

Aim :

To determine the amount of B.O.D. exerted by the given sample(s).

Principle

The Biochemical Oxygen Demand (B.O.D.) of sewage or of polluted water is the amount of oxygen required for the biological decomposition of dissolved organic matter to occur under aerobic condition and at the standardized time and temperature. Usually, the time is taken as 5 days and the temperature 20°C as per the global standard.

The B.O.D. test is among the most important method in sanitary analysis to determine the polluting power, or strength of sewage, industrial wastes or polluted water. It serves as a measure of the amount of clean diluting water required for the successful disposal of sewage by dilution. The test has its widest application in measuring waste loading to treatment plants and in evaluating the efficiency of such treatment systems.

The test consists in taking the given sample in suitable concentrations in dilute water in B.O.D. bottles. Two bottles are taken for each concentration and three concentrations are used for each sample. One set of bottles is incubated in a B.O.D. incubator for 5 days at 20°C; the dissolved oxygen (initial) content (D1) in the other set of bottles will be determined immediately. At the end of 5 days, the dissolved oxygen content (D2) in the incubated set of bottles is determined.

Then m.g./L B.O.D. $\frac{(D - D_2)20}{D_1}$

where,

P = decimal fraction of sample used.

D1= dissolved oxygen of diluted sample (mg/L), immediately after preparation. D2= dissolved oxygen of diluted sample (mg/L), at the end of 5 days incubation

Among the three values of B.O.D. obtained for a sample select that dilution showing the residual dissolved oxygen of at least 1 mg/L and a depletion of at least 2 mg/L. If two or more dilutions are showing the same condition then select the B.O.D. value obtained by that dilution in which the maximum dissolved oxygen depletion is obtained.

Apparatus

1. B.O.D. bottles 300 mL capacity
2. B.O.D. incubator
3. Burette
4. Pipette
5. Air compressor
6. Measuring cylinder etc.

Reagents

1. Distilled water
2. Phosphate buffer solution
3. Magnesium sulphate solution
4. Calcium chloride solution
5. Ferric chloride solution
6. Acid and alkali solution
7. Seeding
8. Sodium sulphite solution
9. Reagents required for the determination of D.O.

Procedure

1. Place the desired volume of distilled water in a 5 litre flask (usually about 3 litres of distilled water will be needed for each sample).
2. Add 1mL each of phosphate buffer, magnesium sulphate solution, calcium chloride solution and ferric chloride solution for every litre of distilled water.
3. Seed the sample with 1–2 mL of settled domestic sewage.
4. Saturate the dilution water in the flask by aerating with a supply of clean compressed air

or at least 30 minutes.

5. Highly alkaline or acidic samples should be neutralised to pH 7.

6. Destroy the chlorine residual in the sample by keeping the sample exposed to air for 1 to 2 hours or by adding a few mL of sodium sulphite solution.

7. Take the sample in the required concentrations. The following concentrations are suggested:

Strong industrial waste	: 0.1, 0.5 and 1 per cent
Raw and settled sewage	: 1.0, 2.5 and 5 per cent
Oxidised effluents Polluted	: 5, 12.5 and 25 per cent
river water	: 25, 50 and 100 per cent

8. Add the required quantity of sample (calculate for 650 mL dilution water the required quantity of sample for a particular concentration) into a 1000 mL measuring cylinder. Add the dilution water up to the 650mL mark.

9. Mix the contents in the measuring cylinder.

10. Add this solution into two B.O.D. bottles, one for incubation and the other for determination of initial dissolved oxygen in the mixture.

11. Prepare in the same manner for other concentrations and for all the other samples

12. Lastly fill the dilution water alone into two B.O.D. bottles. Keep one for incubation and the other for determination of initial dissolved oxygen.

13. Place the set of bottles to be incubated in a B.O.D. incubator for 5 days at 20°C. Care should be taken to maintain the water seal over the bottles throughout the period of incubation.

14. Determine the initial dissolved oxygen contents in the other set of bottles and note down the results.

15. Determine the dissolved oxygen content in the incubated bottles at the end of 5 days and note down the results.

16. Calculate the B.O.D. of the given sample.

Note: The procedure for determining the dissolved oxygen content is same as described in the

experiment under “Determination of dissolved oxygen”.

Observation:

Sample No. Or Description	Concentration	Dissolved oxygen content mg/L				B.O.D. mg/L (5 days 20 ⁰ C)
		Initial D1		Initial D2		
		Bottle No.	D.O. value	Bottle No.	D.O. value	

Results

Sample No.	C.O.D. in mg/L

Experiment No-12

Determination of C.O.D in Water

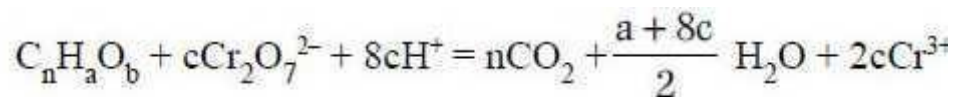
Aim

To determine the Chemical Oxygen Demand (C.O.D.) for given sample.

Principle

Potassium dichromate is a powerful oxidising agent in acidic medium and is obtained in high state of purity.

The reaction involved is:



where, $c = 2/3n + a/6 - b/3$

C.O.D. results are reported in terms of mg of oxygen. N/8 or 0.125 N solution of oxidising agent is used in the determination. Normality double the strength is used. This allows the use of larger samples. Thus, each ml of 0.25 N solution dichromate is equivalent to 2 mg of oxygen. An excess of oxidising agent is added, the excess is determined by another reducing agent such as ferrous ammonium sulphate. An indicator ferroin is used in titrating the excess dichromate against ferrous ammonium sulphate. Blanks are used also treated and titrated to get the correct value of C.O.D.

Apparatus

1. Reflux apparatus
2. Burettes
3. Pipettes

Reagents

1. Standard potassium dichromate solution 0.25N.

2. Sulphuric acid reagent.
 3. Standard ferrous ammonium sulphate.
 4. Ferroin indicator solution.
 5. Mercuric sulphate.
 6. Sulphuric acid crystals.
1. **Standard potassium dichromate solution 0.25 N:** Dissolve 12.259 g $K_2Cr_2O_7$ primary standard grade previously dried at $103^\circ C$ for 2 hours and dilute to 1 litre.
 2. **Sulphuric acid reagent:** Concentrated H_2SO_4 containing 22 g silver sulphate per 4 kg bottle. Dissolve 22 g Ag_2SO_4 in 4 kg bottle and keep it for 2 days. This is the reagent.
 3. **Standard ferrous ammonium sulphate 0.1 N:** Dissolve 39 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 mL conc. H_2SO_4 and cool and dilute to 1 litre. Standardise this against the standard dichromate solution. Dilute 10 mL standard $K_2Cr_2O_7$ solution to about 100 mL. Add 30 mL conc. H_2SO_4 and cool. Titrate with ferrous ammonium sulphate titrant using 2-3 drops of ferroin indicator.

Procedure

1. Place 50.0 mL of sample in a 500 mL refluxing flask.
2. Add 1g mercuric sulphate and a few glass beads.
3. Add sulphuric acid to dissolve the mercuric sulphate and cool.
4. Add 25.0 ml 0.25 N potassium dichromate solution and mix well.
5. Attach the flask to the condenser and start the cooling water.
6. Add the remaining acid reagent (70 mL) through the open end of condenser and mix well.
7. Apply heat and reflux for 5 hours.
8. Cool and wash down the condenser with distilled water.
9. Dilute the mixture to about twice its volume and cool to room temperature.
10. Titrate the excess dichromate with standard ferrous ammonium sulphate using ferroin indicator (2 to 3 drops).

11.Thecolour change from blue green to reddish indicates the end point.

12.Reflux in the same manner a blank consisting of distilled water of equal volume as that of the sample.

Observation

	Burette reading		Volume of ferrous ammonium sulphate
	Initial	Final	
Sample			
Blank			

Calculation

$$\text{mg/L C.O.D.} = \frac{(V1 - V2) N \times 8000}{V}$$

where,

V1 = mL ferrous ammonium sulphate used for blank

V2 = mL ferrous ammonium sulphate used for sample

N = normality of ferrous ammonium sulphate

V = volume of sample used.

Results:

Sample no.	C.O.D. in mg/L

Experiment No-13

Test for Coliforms in Water

Aim

To find the Most Probable Number (MPN) of bacterial density by E.coli test.

Principle

Coliform group comprises of all the aerobic, facultative and anaerobic gram-negative non-spore forming rod shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C. The standard test for this group may be carried out either by multiple tube fermentation technique or by membrane filter technique. The E.coli test by multiple tube fermentation technique consists of 3 phases – presumptive, confirmed and completed.

Escherichia coli (*E.coli*) for the purpose of sanitary examination of water, is defined as a gram-negative, nonspore forming rod which is capable of fermenting lactose with the production of acid and gas at 35°C in less than 48 hours, which produces indole peptone water containing tryptophan, which is incapable of utilising sodium citrate as its sole source of carbon, which is incapable of producing acetyl methyl carbinol, and which gives a positive methyl red test. The results are expressed in terms of MPN (Most Probable Number), which is based on certain probability formulae. The estimate may give a value greater than the actual number of coliform present. The accuracy of any single test depends on the number of tubes fermented. This method helps in describing the sanitary quality of water.

The safety of the water is generally judged from the knowledge of sanitary condition and mentioned by the number of samples yielding positive or negative results. If more than 95% should yield negative results, the safety is usually assured. The scheme of the MPN test is given as follows:

Apparatus

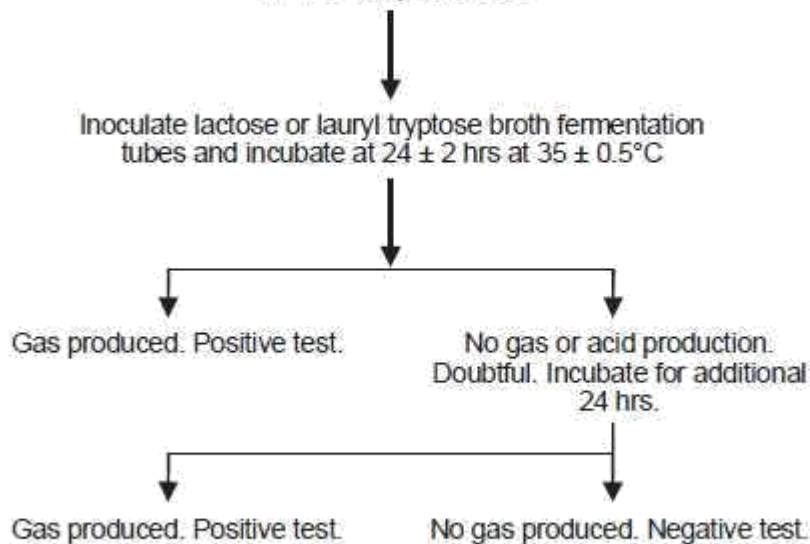
1. Fermentation tubes
2. Petri dishes
3. Autoclave
4. Incubator
5. Test tubes
6. Pipettes
7. Measuring jars
8. Inoculating equipments
9. Media preparation utensils etc.

Reagents

1. Lactose broth
 2. Lauryl tryptose broth
 3. Brilliant green lactose bile broth
 4. Endo agar
 5. Eosin methylene blue agar etc.
1. **Lactose broth:** Beef extract 3 g, peptone 5 g, lactose 5 g and reagent grade distilled water 1 litre. Add these ingredients to reagent grade distilled water, mix thoroughly and heat to dissolve. pH should be 6.8-7.0 after sterilisation.
 2. **Lauryl tryptose broth:** Tryptose 20 g, lactose 5 g, K_2HPO_4 2.75 g, KH_2PO_4 2.75 g, NaCl 5 g, sodium lauryl sulphate 0.1 g, reagent grade distilled water 1 litre, sterilise and use. Add dehydrated ingredients to water, mix thoroughly and heat to dissolve. pH should be 6.8 ± 2 after sterilisation.
 3. **Endo agar:** Peptone 10 g, lactose 10 g, K_2HPO_4 3.5 g, agar 15 g, sodium sulphite 2.5 g, basic fuchsin 0.5 g, distilled water 1 litre, pH 7.4 after sterilisation.
 4. **EMB agar:** Peptone 10 g, lactose 10 g, K_2HPO_4 2 g, agar 15 g, eosin 0.4 g, methylene blue 0.065 g, distilled water 1 litre, pH should be 7.1 after sterilisation.
 5. **Brilliant green lactose bile broth:** Peptone 10 g, lactose 10 g, oxgall 20 g, brilliant green 0.0133 g, distilled water 1 litre, pH should be 7.2 after sterilisation and is then ready for use. Store away from direct sunlight to extend the reagent stability to 6 months.

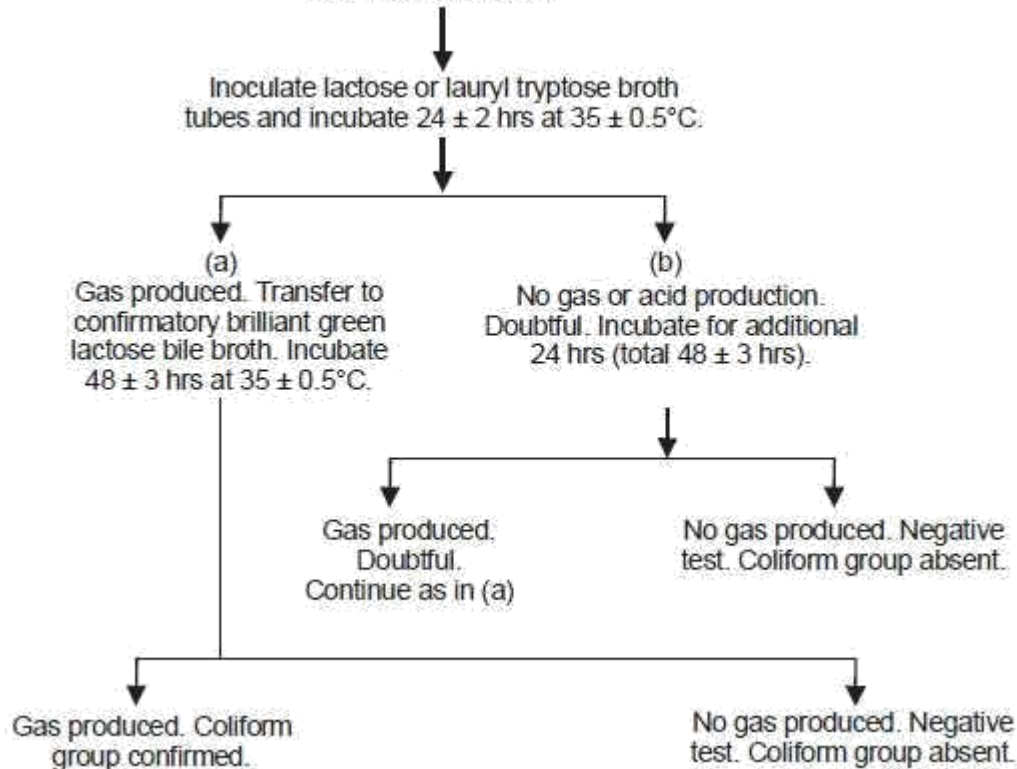
SCHEMATIC OUTLINES FOR THE E.COLI TEST PROCEDURE

1. Presumptive test

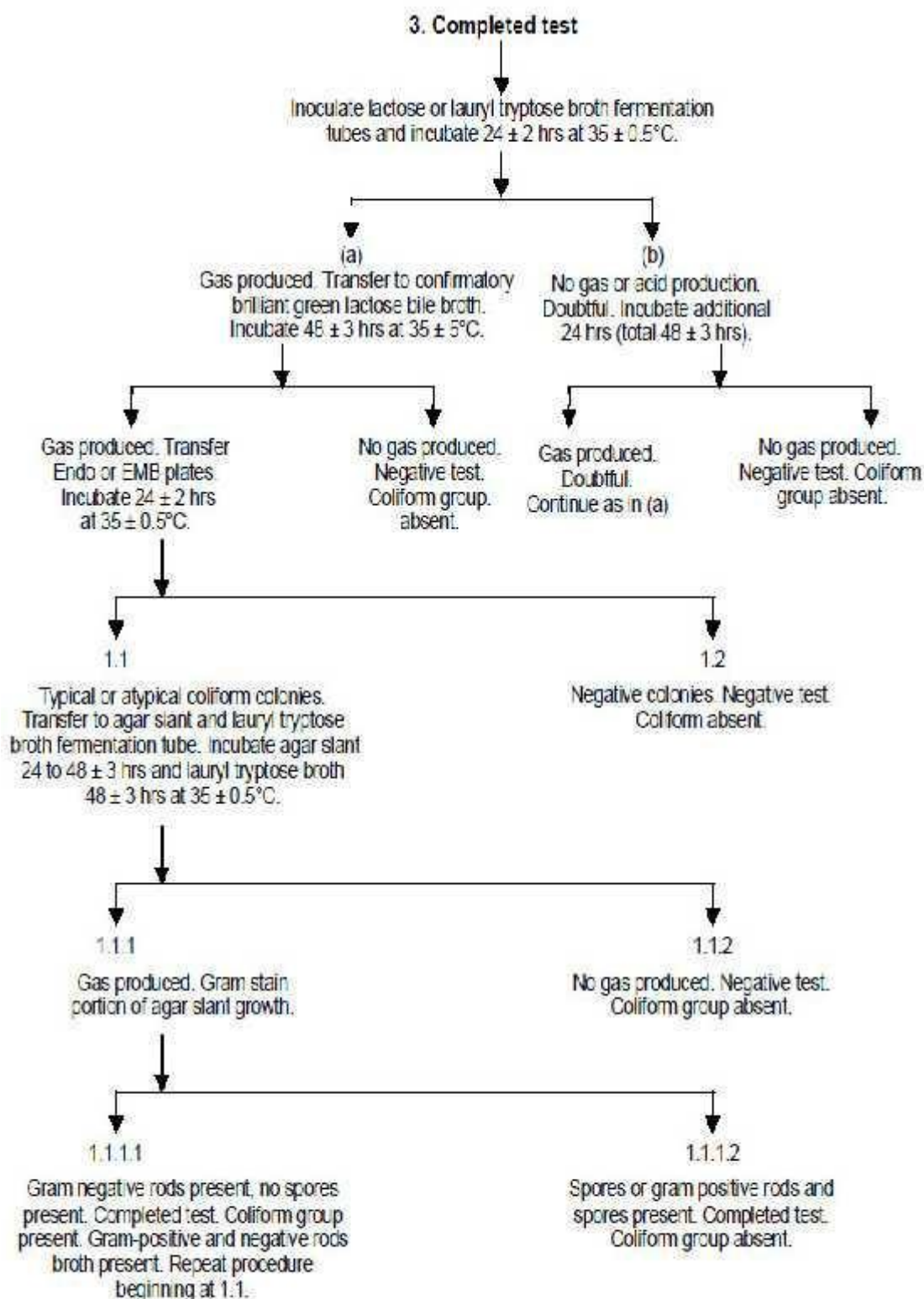


Schematic outline of presumptive test for coliform detection.

2. Confirmed test



Schematic outline of presumptive, confirmed and completed test for total coliform detection.



Procedure

General

Clean and sterilise all the glasswares.

Presumptive Test

1. Inoculate a series of fermentation tubes with appropriate graduated quantities (multiples and sub-multiples of 10) of the water to be tested. The concentration of nutritive ingredients in the mixture of the medium should conform to the specifications. The partitions of the water sample used for inoculating lactose or lauryl tryptose broth fermentation tubes will vary in size and number with the character of the water under examination. Usually, decimal multiples and sub-multiples of 1mL of the sample are selected. Inoculate 10 mL portion of each water sample provided into different one of the three large tubes containing 10 mL of lactose or lauryl tryptose broth which has been prepared with twice the normal concentration of constituent to allow for dilution. Inoculate 1.0 mL and 0.1 mL of water into small tubes (two sets of three each) of single strength lactose or lauryl tryptose broth.
2. Incubate the inoculated fermentation tubes at $35\pm0.5^{\circ}\text{C}$. At the end of 24 ± 2 hrs shake each tube gently and examine and if no gas is formed, repeat this test at the end of 48 ± 3 hrs.
3. Record the presence or absence of gas formation at each examination of the tubes. Formation within 48 ± 3 hrs of gas in any amount in the inverted fermentation tubes constitutes a positive presumptive test. Active fermentation may be shown by the continued appearance of small bubbles of gas throughout the medium outside the inner vial in the fermentation tubes. Presumptive test without confirmation should not be used routinely except in the analysis of heavily polluted water, sewage or other

waste, which are not suitable for drinking purpose.

Confirmed Test

1. Lactose or lauryl tryptose broth may be used for primary fermentation in presumptive test to avoid false positive results.
2. Brilliant green lactose bile broth fermentation tubes are used in confirmed test.
3. Submit all primary fermentation tubes showing any amount of gas at the end of 24 hrs incubation to the confirmed test.
4. Gently shake primary fermentation tube showing gas formation and with a sterile metal loop, transfer one loop full of medium to a fermentation tube containing brilliant green lactose bile broth.
5. Incubate the inoculated brilliant green lactose bile broth tube for 48 ± 3 hrs at $35 \pm 0.5^\circ\text{C}$.
6. The formation of gas in any amount in the inverted vial of the brilliant green lactose bile broth fermentation tube at any time within 48 ± 3 hrs constitutes a positive confirmed test.
7. If no gas is formed, it is a negative confirmed test and E.coli is absent.

Completed Test

Completed test is the next step following the confirmed test. It is applied to the brilliant green lactose bile broth fermentation tubes showing gas in the confirmed test.

1. Streak one or more endo or Eosin Methylene Blue (EMB) agar plates (taken in Petri dishes) from each tube of brilliant green lactose bile broth showing gas.
2. While streaking it is essential to ensure the presence of some discrete colonies separated by at least 0.5 cm from one another.
3. Insert the end of the streaking needle into the liquid in the tube to a depth of 5mm.
4. Streak the plate by bringing only the curved section of the needle in contact with

the agar surface so that the latter will not be scratched or torn.

5. Incubate the Petri dishes (inverted) at $35 \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hrs.
6. The colonies developing on endo or eosin methylene blue agar may be typical (unnucleated, with or without metallic sheen) atypical (opaque, unnucleated, mucoid, pink after incubation for 24 hrs) or negative (all others).
7. From each of these plates fish out one or two colonies and transfer to lauryl tryptose broth fermentation tubes and to nutrient agar slants.
8. Incubate the secondary broth tubes and agar slants at $35 \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hrs or 48 ± 3 hrs and if gas is not produced in 24 hrs gram stained preparation from these agar slant cultures are made.
9. The gas formation in the secondary lauryl tryptose broth tubes and the demonstration of gram-negative non-spore forming rod shaped bacteria in agar culture may be considered a satisfactory positive completed test.
10. If after 48 ± 3 hrs gas is produced in the secondary fermentation tubes and no spore of gram positive rod are found on the slant, the test may be considered as positive completed test and this demonstrates the presence of coliform organisms.

Differentiation of *E. coli* and *A. aerogenes* on eosin or EMB agar can be done by referring the following table.

Differentiation of *E.coli* and *A. aerogenes* on eosin or EMB agar

Characteristic	<i>Escherichia coli</i>	<i>Aerobacter aerogenes</i>
Size	Well isolated colonies are 2-3mm in diameter.	Well isolated colonies, are larger than those of <i>E. coli</i> usually 4-6 mm in diameter.
Confluence	Neighbouring colonies show little	Neighbouring colonies run together tendency to run together. quickly.
Elevation	Colonies are slightly raised, surface is flat or slightly concave, rarely convex.	Colonies are considerably raised and marked by convex, occasionally centre drops precipitely.
Appearance by transmitted light	Dark, almost black centre that extend across more than three fourths of the diameter of the colony, internal structure of central dark portion is difficult to discern.	Centres are deep brown not as dark as those of <i>E. coli</i> and smaller in proportion to the rest of the colony. Striated internal structure is often observed in young colonies.
Appearance by reflected light	Colonies are dark, button like, often concentrically ringed with a greenish metallic sheen.	Much lighter than <i>E. coli</i> , metallic sheen is not observed except in a depressed center.

Gram Staining

Reagents

1. Ammonium oxalate-crystal violet (Hucker's)
2. Lugol's solution
3. Counter stain
4. Acetone alcohol.

Procedure

1. Prepare a light emulsion of the bacterial growth on an agar slant in a drop of distilled water on a glass slide.
2. Air-dry or fix by passing the slide through a flame and stain for 1minute with ammonium oxalate-crystal violet solution.
3. Rinse the slide in tap water and then apply Lugol's solution for 1minute.
4. Rinse the stained slide in tap water.
5. Decolorise with acetone alcohol till the stain is just removed.
6. Counter-stain with safranin for 15 seconds and then rinse with tap water.
7. Blot dry with blotting paper and view through the microscope.
8. Cells that decolorise and accept the safranin stain are pink and are defined as gram negative. Cells that do not decolorise but retain the crystal violet stain (deep blue) are defined as gram positive.

Steps in the gram staining are shown in the following table.

Step	Procedure	Results	
		Gram + ve	Gram - ve
Initial stain	Crystal violet for 30 sec.	Stains purple	Stains purple
Mordant	Iodine for 30 sec.	Remains purple	Remains purple
Decolonisation	95% ethanol for 10-20 sec.	Remains purple	Becomes colourless
Counter stain	Safranin for 20-30 sec.	Remains purple	Stains pink

The number of positive finding of coliform group organisms resulting from the multiple portion decimal dilution planting should be computed as the combination of positives and recorded in terms of the Most Probable Number (MPN). The MPN for the variety of planting series are presented in table in Appendix III. The values are at the 95% confidence limit for each of the MPN determined. These values are prepared for 10,1 and 0.1mL combination. If the combination is 100, 10, 1mL, the MPN is 0.1 times the value in the table. If on the other hand a combination corresponding to 1, 0.1, and 0.01 mL is planted, record MPN as 10 times the value shown in the table.

$$\text{MPN/100 mL} = \frac{\text{No. of positive tubes} \times 100}{\sqrt{\text{mL sample in negative tubes} \times \text{mL sample in all tubes}}}$$

Sample no. or description	Date and time of observation	Date and time of incubation	Results after incubation for various volumes of samples inoculated (mL) + ve or - ve									Testcase
			10	10	10	1	1	1	0.1	0.1	0.1	
												Presumptive test 24 hrs
												Presumptive test 48 hrs
												Confirmed test 48 hrs
												Completed test 24 hrs
												Completed test 48 hrs
Number of + ve tubes												
mL of sample in - ve tubes												

Calculation

☐ **Case (i)**

For three each of 10 mL, 1 mL and 0.1 mL sample concentration combinations
MPN from the MPN table (**Appendix-III**) =

☐ **Case (ii)**

For other combinations and dilutions

$$\text{MPN/100 mL} = \frac{\text{No. of positive tubes} \times 100}{\sqrt{\text{mL sample in negative tubes} \times \text{mL sample in all tubes}}} = \dots\dots\dots$$

Result:

MPN/100 mL =

Viva questions:

1. Determine the importance of BOD?
2. what is the significance of BOD and COD in environmental engineering lab?