MANUAL&WORK BOOK OF

ENVIRONMENTAL ENGINEERING LABORATORY

Name: ……

Subject: …… Roll No.:……..

Year:…….. Branch: …… Sec: ..……

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CONTENTS

LIST OF EXPERIMENTS

1. **Determination of pH and Turbidity**
2. **Determination of conductivity and total dissolved salts**
3. **Determination of Alkalinity/Acidity**
4. **Determination of Chlorides**
5. **Determination and Estimation of total solids, organic solids and inorganic solids**
6. **Determination of Iron**
7. **Determination of Dissolved oxygen**
8. **Determination of Nitrogen**
9. **Determination of total phosphorous**
10. **Determination of B.O.D.**
11. **Determination of C.O.D.**
12. **Determination of optimum coagulant dose**
13. **Determination of chlorine demand**
14. **Presumptive coli form test**

EXPERIMENT: 1 **DATE:**

pH

Aim: To determine the pH of the given water sample.

Introduction:

Measurement of pH is one of the most important and frequently used tests in water and wastewater analysis. Practically every phase of water and wastewater treatment. E.g., acid – base neutralization, water softening, precipitation, coagulation, disinfection and corrosion is pH dependent

pH of aqueous solution can defined as negative logarithm of hydrogen ion concentration. pH values from 0 to 7 are considered to be diminishing acidity, 7 to 14 increasing alkalinity and pH, 7 is considered to be neutral. It is a measure of acid-base equilibrium. At a given temperature, the intensity of acidic/basic character of a solution is indicated by the hydrogen ion activity. Alkalinity and acidity are the acid – base neutralization capacities of water and usually are expressed as mg/I of $CaCO_3$.

pH is defined by Sorenson is ---------- - log(H+)

It is intensity factor of acidity

In natural waters, the pH value is governed by the carbon dioxide/bicarbonate/carbonate equilibrium. It may be affected by humic substances by changes in the carbonate equilibria due to the bioactivity of plants and in some cases by hydrolysable salts. The effect of pH on the chemical and biological properties of liquids makes its determination very important. It is used in several calculations in analytical work and its adjustment is necessary for analytical procedures.

The determination of pH by conventional chemical means is not practicable and the equilibria which are involved depend to some extent on temperature. The precise accepted scale of pH must therefore be beased on an agreed primary standard. The calorimetric indicator methods can be used only if approximate values are required.

The pH determination is usually done by electrometric method which is the most accurate method and free of interferences.

Electrometric method:

The pH is determined by measurement of the electromotive force of a cell comprising an indicator electrode (an electrode responsive to hydrogen ions such as glass electrode) immersed in the test solution and a reference electrode is usually achieved by means of a liquid junction, which forms a part of the reference electrode. The emf of this cell is measured with pH meter. This is a impedance electrometer calibrated in terms of pH.

Apparatus:

Glass Electrode:

This must be compatible with the pH meter used and must be suitable for the particular application. Special electrodes are available for pH values greater than 10 and for use at temperatures greater than 60^0C.Combined glass/reference electrodes are also available and are convenient to use.

Reference Electrode:

The mercury/calomel electrode is widely used but the silver/silver chloride electrode may be preferable on account of it being more reproducible and more reliable. Less concentrated solutions of KCL are more preferable as filling solutions than the saturated solution often used because problems due to clogging of the electrode or the liquid junction will be avoided. To prevent dissolution of the silver chloride film, the potassium chloride filling solution of Ag/agCl electrodes should be saturated with AgCl.

pH meter:

Both mains and battery operate models are available;the later type can be used for fields measurements. The most accurate pH meters can be read to better than±0.005 ph. Unit.

Reagents:

Buffer solutions for pH4, pH7, pH9.2.

Note: In general analytical reagent grade chemicals are satisfactory for the preparation of this solution. Commercial buffer tablets are available in the market for the preparation of solution of above pH. Values (each tablet dissolved in 100 ml give the buffer solution of required pH).

Procedure:

1. Standardize the pH meter according to the manufacturere's instructions.

2. Select a standard buffer solution with a pH value close to that of the water to be treated.
3. Set the temperature control to the temperature of the buffer.
4. Set the meter to the pH of the bnuffer at the temperature.
5. Check the electrode response by measuring a second standard buffer solution of different pH.
6. Wash the electrode thoroughly first with distilled water and then with the sample.
7. Set the temperature control to the temperature of sample.
8. Immerse electrodes in the sample and record the pH after stabilizing the system.

Note: Between measurements, the electrodes are kept in distilled water new or dried out glass electrodes should be prepared for the use by soaking in 0.1 N HCl for 8 hours or according to the maker's instructions.

Result:

Significance:

pH (6.5 to 8.5) has no direct adverse effect on health, however a lower value below 4 with produce sour taste and higher value above 8.5 a bitter taste. Higher values of p^H hasten the scale formation in water heating apparatus and also reduce the germicidal potential of chlorine. High p^H induces the formation of trihalomethanes which are causing cancer in human beings.

According to BIS, water for domestic consumption should have a pH between 6.5 to 8.5.

Applications:

1. Determination of p^H is one of the important objectives in biological treatment of the waste waters. In anaerobic treatment, if the p^H goes below 5 due to excess accumulation of acids, the process is severely affected. Shifting of p^H beyond 5 to 10 upsets the aerobic treatment of the waste waters. In these circumstances, the p^H can be adjusted by addition of suitable acid or alkali to optimize the treatment of the waste waters.
2. p^H value or range is of immense vale for any chemical reaction. A chemical shall be highly effective at a particular p^H.

3. Dewatering of sludges, oxidation of cyanides and reduction of hexavalent chromium into trivalent chromium also need a favourable pH range.
4. It is used in the calculation of carbonate, bicarbonate, CO_2 corrosion, stability index and acid base euqlibria.

DETERMINATION OF TURBIDITY

AIM: To determine the turbidity of the given water samples in NTU using Nephelometer.

Principle:

Clarity of water is an important aesthetic consideration for acceptability by the consumer and also necessary requirement in many manufacturing processes. Water treatment plants treating surface waters commonly relay on particles or solids removal through coagulation, flocculation, sedimentation, followed by filtration (rapid gravity filters) or plain sedimentation followed by filtration (slow sand filtration). These treatment processes improve the water quality in terms of water clarity and bacterial quality, to make water potable and aesthetically acceptable to consumer and also to meet industrial water quality requirements. Turbidity is one of the indicators used to assess the quality of water.

Generally turbidity in surface water is caused by suspended and colloidal solids like clay, silt, finally divided organic and inorganic matter, planton & other microscopic organisms.

1. Turbidity is the measure of relative water clarity. It is not colour.
2. Turbidity is defined as an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through the samples.
3. Turbidity is the haziness or cloudiness of a fluid caused by suspended solids that are generally invisible to naked eye (clay, silt, finely divided organic matter, algae).
4. Turbidity is a measure of the degree to which water looses its transparency due to presence of suspended, colloidal & dissolved particles or solids.

The world Health organization (WHO), recommended that turbidity of drinking water
Should not be more than 5 NTU and ideally be below 1 NTU.

The sediments from erosion, urban runoff, phyto – plankton, recirculation of bottom sediments causes turbidity in water.

1. The suspended particles absorbs heat from sunlight, makes water warmer and thus reduces the saturated dissolved oxygen (DO) level. Warm wter affects aquatic organisms.
2. Turbidity reduces the reservoir capacity due to suspended solids sedimentation.
3. Due to turbidity the suspended particles scatter the light and decreases the photosynthetic acitivity of plants and algae results in the lowering of DO.

Some of the impacts of turbidity:

1. Turbid water would become aesthetically unacceptable as body would like to drink turbid water and also effects the industrial processes.

2. Turbidity is measured to assess the performance of water treatment plants.

Turbidity is generally measured by the turbidity meter instrument or Nephelometer instrument.

1. Turbidity generally can be measured either by its effect on the transmission of light which is termed as turbidimetry (or) by its effect on the scattering of light which is termed as Nephelometry.
2. Turbidity meter can be used for sample with moderate turbidity and nephlometer is used for smaples with low turbidity.
3. The turbidity is measured in Nephelometric turbidity units (NTU).
4. The instrument is known as Nephelometric turbidity meter, measures the intensity of light scattered at 90° as beam of light passes through water samples.

Apparatus: Nephelometer, waters, standard volumetric flasks(100ml), pipette, measuring cylinder, beakers.

Chemicals: Hydrazine sulphate, Hexamethylene tetramine, distilled water.

Procedure:

A. Preparation of solution I: Dissolve I gm of Hydrazome sulphate in the little amount of distilled water and later make up to 100ml with distilled water in a standard volumetric flask.
B. Preparation of solution II : Dissolve 10gms of hexamethylene tetramine in the little amount of distilled water & then make up to 100ml with distilled water in a standard volumetric flask.
C. Preparation of solution III : Mix 5ml of solution – I and 5ml of solution –II. Allow to stand the mixture solution for 24hrs at 25° ± 3°c, after that make up the mixture solution (10ml) up to 100ml by using standard volumetric flask. This solution have a turbidity of 400NTU.
D. Preparation of solution IV : Pipette out about 10ml of solution III and make up to 100ml with distilled water by using standard volumetric flask. Now this solution have the turbidity about 40NTU.

Note: Stock solution or suspension is stable for one year if properly stored and use high quality of distilled water.

According to standard methods for the examination of water & waste water, 40NTU has an approximate turbidity of 40 Jackson Turbidity Units (JTU) when measured on the candle turbidity meter but not identical to them.

1 NTU approximately equal to 1 JTU.

Procedure:

1. Switch on Nephelometric turbidity meter and wait few minutes till it warm – up.
2. Insert the distilled water cuvette in to the Nephelometer chamber and close the cover. Use the set Zero (0) by using potentiometer knob to set the display to zero (0).
3. Replace distilled water with another cuvette containing the standard 100NTU solution.
4. Theoretically this would ensure correct display for subsequent samples.
5. However, many standards of intermediate solutions need to be prepared and tested before testing any number of samples.
6. After testing sufficient number of intermediate standard (say about 6 to 8 standard solutions) solutions, now test the test samples and note the displayed values of turbidity.

Observations:

Readings are noted in the table

S.No.	Test Samples	Nephelometer NTU readings	Remarks
1			
2			
3			
4			
5			

Results:

EXPERIMENT: 2 **DATE:**

DETERMINATION OF CONDUCTIVITY

AIM: To determine the conductivity of the given water sample.

Apparatus: Conductivity meter, thermometer, wash bottle, beakers, standard volumetric flask (100ml).

Chemicals: Collected water samples, distilled water, potassium chloride, tissue papers.

Theory: Specific conductance is determined by using a wheat stone bridge in which variable resistance is adjusted. So that it is equal to the resistance of the unknown solution between the electrodes of a standard conductivity cell.

The cell constant is determined experimentally with a standard solution of known conductance. Note the cell constant value.

Interference:

1. Dissolved CO2 increases conductivity without increasing the mineral salt content. Hower the effect is not large and it is usual to ignore it.
2. Temperature affects the conductivity, which varies about 2% per degree Celsius. The temperature of 25°C is taken as standard.
3. In low pH water H+ ions and in high pH water OH- ions, may contribute substantially to conductivity owing to high equivalent conductivity of these ions.
4. It is not convenient to use water containing large amount suspended matter.
5. Highly suspended matter also affects the electrical conductance values.
6. Water samples containing fat, grease, oil, tar etc., may contaminate the electrodes causing wrong & erratic results.

Procedure:

1. Calibration should be carried out after allowing 15 minutes warm – up time for the instrument.
2. The electrodes should always be immersed in the distilled water. If the electrode is in dry position, soak it in distilled water before use.
3. Select 1 for cell constant & choose the appropriate conductivity range.
4. Select the calibration position in this setting; immerse the electrode in the standard 0.01 NKcl solution. Adjust the calibration knob till the display reads I and 000. The position of

1 varies with the range selected. So it is immaterial. The temperature knob should be set 25°C during this operation.

5. After calibration, change the selection switch to measure (or) conductivity read position.
6. Rinse the electrode with distilled water and clean with the filter paper.
7. Immerse the electrode in the test solution and select the temperature knob to 25°C.
8. After the reading stabilizes, Value may be noted. The values may be in the particular units. i.e., may be milli (or) micro Siemens.
9. If a single 'I' appears on the screen, it indicates over – range. Then select the next higher valaue range till readings appears on the display screen.

Note down the displayed values in the given table.

S.No.	Test sample	Temperature	Conductivity
1			
2			
3			
4			
5			

Result:

EXPERIMENT: 3 **DATE:**

ALKALINITY

Aim: To determine the Alkalinity of the given water sample.

Introduction

The alkalinity of water is a measure of its capacity to neutralize acids. The alkalinity of natural waters is due to the salts of carbonates, bicarbonates, borates, silicates and phosphates along with the hydroxyl ions if free state. However the major portion of the alkalinity in natural waters is caused by hydroxide, carbonate and bicarbonates which may be ranked in order of their association with high pH values. Alkalinity values provide guidance in applying proper doses of chemicals in water and waste water treatment processes, particularly in coagulation, softening, and operational control of anaerobic digestion.

Principle:

Alkalinity of sample can be estimated by titrating with standard sulphuric acid. Titration to pH 8-3 or decolonization of phenolphthalein indicator will indicate complete neutralization of pH and ½ of CO_3 while pH or sharp change from yellow to pink of methyl orange indicator will indicate total alkalinity (complete neutralization of OH, CO_3, HCO_3).

Interference:

Colour, turbidity, iron and residual chlorine are prime sources of interference. Colour and turbidity can be avoided using potentiometric titrations. Residual chlorine can be removed by adding thiosulphate.

Apparatus: Pipette, burette, conical flask, standard volumetric flasks, beaker, glass rod.

Reagents:

1. Standard H_2SO_4 (0.02 N): Prepare 0.1 N H_2SO_4 by diluting 3.0 ml conc. $H_2SO_4$to 100ml. Standardize it against standard Na_2CO_3 0.1N. Dilute appropriate volume of H_2SO_4 (approximate o.1N) to 1000ml to obtain standard 0.02n H_2SO_4.
2. Phenolphthalein indicator: Dissolve 1 gm of phenolphthalein powder in 100ml of absolute alcohol (95%)
3. Methyl orange indicator: Dissolve 1 gm of methyl orange indicator in 100ml of absolute alcohol (95%)

Procedure:

1. Take 25 or 50 ml sample in a conical flask and add 2-3 drops of phenolphthalein indicator.
2. If pink colour develops titrate with 0.02 N H_2SO_4 till it disappears or pH is 8.3. Note the volume of H_2SO_4required.
3. Add 2-3 drops of methyl orange to the same flask, and continue titration till pH down to 4.5 or orange color changes to pink.
   - Note the volume of H_2SO_4
4. In case pink color does not appear after addition of phenolphthalein continue as in 3 above.
5. Calculate Total (T) phenolphthalein (P) and methyl orange alkalinity as follows and express in mg/I as $CaCO_3$.

Observations:

Sample Details	Volume of Sample (ml)	Phenolphthalein			Methyl Orange		
		Initial Burette Reading (ml)	Final Burette Reading (ml)	Volume of H_2SO_4 Consumed (ml)(A)	Initial Burette Reading (ml)	Final Burette Reading (ml)	Volume of H_2SO_4 Consumed (ml)(B)
1.							
2.							
3.							
4.							
5.							

Calculations:

P – Alkalinity mg/l, as $CaCo_3$ = A x 1000/ml sample.

MO – Alkalinity, mg/l as $CaCo_3$ = B x 1000/ml sample.

T – Alkalinity, mg/l as $CaCo_3$ = (A+B) x 1000/ml sample.

In case H_2SO_4 is not 0.02 N apply the following formula:

Alkalinity, Mg/ l as $CaCo_3$ = $\frac{A \times N \times 50000}{\text{Ml of sample}}$

Where N = normality of H_2SO_4 used.

Once the phenolphthalein and total alkalinity is determined, then three types of alkalinities i.e. hydroxide, carbonate and bicarbonate are easily, calculated from the table given below.

Value of P and T	Alkalinity Due To		
	OH^-	CO_3^-	$H CO_3^-$
P=O	0	0	T
P<1/2 T	0	2P	T-2P
P=1/2 T	0	2P	0
P>1/2 T	2P- T	2 (T-P)	0
P=T	T	0	0

Results:

Significance:

1. Highly alkaline waters are usually unpalatable and consumers tend to seek other supplies.
2. Chemically treated waters sometimes have rather high pH values which have met with some objection on the part of consumers.
3. Large amount of alkalinity imparts a bitter taste to water.

Applications:

1. Chemical Coagulation of water and waste water: To neutralize acids produced during flocculation, the sample should be alkaline as otherwise further floc formation [either $A1(OH)_3$ or $Fe(OH)_3$]slowly ceases.
2. Water Softening: To find out the quantity of lime and soda-ash required for the removal of hardness, alkalinity should be found out.
3. Corrosion control: To control the Corrosion due to acids, natural waters are rendered alkaline.
4. Effluents of waste water: Waste waters containing excess caustic (hydroxide) alkalinity are not to be discharged into natural water bodies or sewers.

Excess alkalinity in water is harmful for irrigation which leads to soil damage and reduce crop yields. Water having an alkalinity content of less than 250mg/I is desirable for domestic consumption.

ACIDITY

Aim: To determine the Acidity of the given water sample.

Introduction:

Acidity of a liquid is its capacity to donate H+ ions. Since most of the natural waters and sewage are buffered by carbon dioxide – bicarbonate system. The acidity present due to free CO_2 has no significance from public health view point. Waters containing mineral acidity (due to H_2SO_4 and HCl) are unacceptable. Further, acid waters pose problem of corrosion and interference in water softening.

Principle:

The mineral acids present and contributing mineral acidity can be calculated by titrating or neutralizing samples to pH 4.3. The CO_2 and bicarbonates (carbonic acid) present in the sample can be neutralized completely by continuing the titration to pH 8.3

Interference:

Color, turbidity, iron, and residual chlorine are prime sources of interference. Colour and turbidly can be avoided using potentiometric titrations. Residual chlorine can be removed by adding sodium thiosulphate.

Apparatus: Pipette, burette, conical flask, standard volumetric flasks, beaker, glass rod.

Reagents:

1. **Standard sodium hydroxide 0.02 N:** Dissolve 0.8 g NaOH and dilute to 1000mL using CO_2 free distilled water. Store in airtight rubber stoppered pyrex/corning glass bottle to protect from atmospheric CO_2.
2. **Phenophthalein indicator:** Dissolve 1 gm of phenolphthalein powder in 100ml of absolute alcohol (95%)
3. **Methyl orange indicator:** Dissolve 1 gm of methyl orange powder in 100ml of absolute alcohol (95%)

Procedure:

1. Measure suitable volume of sample (250(or)50ml) in a conical flask
2. Add 2 drops of methyl orange and titrate with standard 0.02N NaOH till color changes to faint orange, characteristic of pH 4.3 - 4.4

3. Note down the volume of NaOH required.
4. Add 2-3 drops phenolphthalein indicator and continue titration with NaOH till faint pink color appears.
5. Note down the volume of additional NaOH required.

Observations:

Sample Details	Volume of Sample (ml)	Methyl Orange			Phenolphthalein		
		Initial Burette Reading (ml)	Final Burette Reading (ml)	Volume of NaOH Consumed (ml)(A)	Initial Burette Reading (ml)	Final Burette Reading (ml)	Volume of NaOH Consumed (ml)(B)
1.							
2.							
3.							
4.							
5.							

Calculations:

i) Each mL of 0.02 N NaOH = 1 mg of $CaCO_3$

Therefore, acidity mineral or due to CO_2 as mg/L $CaCO_3$ = mL 0.02 N NaOH required x 1000/mL of the sample

ii) In case if normality of NaoH is othr than 0.02 N, follow the calculation given below:

Acidity (Mineral) or due to CO2 as mg/L = A/B x N x 50000/ml of the sample

Where A = NaOH required for sample to raise pH up to 4.4 – 4.3

B = ml NaOH required for sample to raise pH from 4.4 to 8.3

C = normality of NaOH used

Results:

Significance:

1. Acidity interferes in the treatment of water (as in softening).
2. It corrodes pipes (zinc coating of G.I. Pipes get dissolved).
3. Aquatic life will be affected.
4. pH is critical factor for bio-chemical reaction. The favourable pH is 6.8 to 7.5.
5. Waters containing mineral acidity are unpalatable.
6. Waters having acidity more than 50 mg/L cannot be used in RCC works.

Applications:

1. The amount of CO_2 present is an important factor in determining whether removal by aeration or simple neutralization with lime or sodium hydroxide will be chosen as the treatment method.
2. The size of equipment, chemical requirement, storage space and cost of treatment all depend upon amount CO_2 present.
3. CO_2 is an important consideration in estimating chemical requirements for lime or lime soda – ash softening processes.
4. Most industrial wastes containing mineral acidity must be neutralized before they are subjected to biological treatment or direct discharge into water course of sewers.

EXPERIMENT: 4 **DATE:**

CHLORIDES

Aim: To estimate the amount of chlorides present in the given water sample.

Introduction:

Chloride ion is generally present in natural waters. The presence of chloride in natural waters can be attributed to dissolution of salt deposits, discharge of wastewater from industries, oil well operations and sea water instruction in coastal areas. Each of these sources may result in local contamination of both surface and ground water sources.

The salt taste produced by chloride depends on the chemical composition of the water. A concentration of 250ml/I may be detectable in some waters containing sodium ions. On the other hand, the typical salty taste may be absent in water containing 1000mg/I chloride when calcium and magnesium ions are predominant. A high chloride content may harm metallic pipes and structures as well as agricultural pumps.

Four methods are suggested for the estimation of chloride.

- Mercuric nitrate method
- An Argentometric method
- A Potentiometric method
- Ion Chromatographic method

The mercurimetric method is recommended when accurate determination of chloride is required, particularly at low concentration. The peontiometric method is suitable when the sample is colored or turbid. Argentometric method is the simplest and can be a method of choice for variety of samples.

Argentometric method:

Principle:

Chloride is determined in a natural or slightly alkaline solution by titration with standard silver nitrate, using potassium chromate as an indicator. Silver chloride is quantitatively precipitated before red silver chromate is formed. The chemical reactions involved in this method are given below:

$Ag + Cl$ ----------> $AgCl$ (white precipitate)

$2Ag + CrO_4$ ----> Ag_2CrO_4 (red precipitate)

Interferences:

Bromide, iodide and cyanide are measured as equivalent of chloride ions in all the methods suggested except in Chromatographic method. If the sample contains sufficient thiosulfate, thiocyanate, cyanide, sulfite, and sulfide to interfere seriously with the determination, they may oxidize to non-interfering substances as explained below.

Measure a suitable quantity of sample into a conical flask and dilute to 150 ml with water. Add 25ml H_2O_2 (3%) and boil for 15 minutes, then add further 10ml H_2O_2 and boil for 5 minutes. Repeat the same until the solution is thiocynate free.

If the sample is too colored or turbid to allow the end point to be readily detected. This interference may be reduced by the following treatment with a suspension of aluminium hydroxide.

Add 3ml of Aluminium hydroxide suspension to the sample. Stir thoroughly, set aside for a few minutes and filter. Wash the precipitate with distilled water. Collect the washings with the filtrate and continue as described in the procedure.

Apparabus: Pipette, burette, conical flask, standard volumetric flasks, beaker, glass rod.

Reagents:

1) Potassium chromate indicator: Dissolve 50g K_2CrO_4 in distilled water. Add $AgNO_3$ till definite red precipitate is formed. Allow to stand for 12 hrs. Filter and dilute to 1000ml.
2) Silver nitrate – 0.01 N : Dissolve 2.395 g $AgNO_3$ and dilltet to 1000ml
   Standardize against NaCl, 0.141N
   1ml of 0.14 AgNO3 = 0.5mg Cl
3) Sodium chloride, 0.14IN : Dissolve 824.1mg NaCl (dried at 149°C) and dilute to 1000ml, 1ml = 0.5mg Cl
4) Special reagent to remove color and turbidity: Dissolve 125 g AIK $(SO_4)2. 12H_2O$ or $AINH_4(SO_4)_2. 12H_2O$ and dilute to 1000ml. Warm to 60°C and add 55ml of Concentrated NH_3OH slowly. Allow to stand for 1hr. Transfer to large bottle and wash precipitate by successive addition with thorough mixing and decanting with distilled water until free from chloride. When freshly prepared, a suspension occupies a volume of 1 littre approximately.

Procedure:

1) Take 50ml of sample adjusted to pH 7.0-8.0 and add 1ml of K_2CrO_4
2) Titrate with standard $AgNO_3$ solution until to get brick – red colour precipitate.
3) Standardize $AgNO_3$ against standard NaCl
4) For better accuracy titrate distilled water (50ml) in the same way to establish reagent blank.

Observation:

Sample Details	Volume of Sample (ml)	Initial Burette Reading (ml)	Final Burette Reading (ml)	Volume Of $AgNO_3$ Consumed (ml)	Chlorides (mg/I)
1.					
2.					
3.					
4.					
5.					

Calculation:

$$\text{Chloride mg/L} = \frac{(A-B) \times N \times 35.45 \times 1000}{\text{Ml of sample}}$$

Where A = ml $AgNO_3$ required for the sample

B=ml $AgNO_3$ required for the blank and

N=Normality of $AGNO_3$ used

Result:

Concentration levels of Chlorides in the samples are

Significance:

Chlorides associated with sodium exerts salty taste, when its concentration is more than 250mg/I. There is no known evidence that chlorides constitute any human health hazard. For this reason, chlorides are generally limited to 250mg/I in supplies intended for public use. In many areas of the world where water supply is scarce, sources containing as much as 2000mg/I are sued for domestic purposes without the development of adverse effects, once the human system becomes adopted to the water.

It can also corrode concrete by extracting calcium in the form of calcide. Magnesium chlorides in water generate hydrochloric acid after heating which is also highly corrosive and create problems in boilers.

Applications:

1. Chlorides determination in natural waters is useful in the selection of water supplies for human use.
2. Chlorides determination is used in determining the type of desalting apparatus to be used.
3. The chloride determination is used in control pumping of ground water from locations where intrusion of sea water is a problem.
4. Chlorides interfere in the determination of chemical oxygen demand (COD). A correction must be made on the basis of the amount of chlorides present.

EXPERIMENT: 5 **DATE:**

Determination of total solids

AIM: To determine the total solids present in the given water samples.

APPARATUS: China dish, Beaker, glass rod, measuring cylinder, water bath, hot air oven.

CHEMICALS: Given water samples in the Lab.

DESCRIPTION:

Solid, considered to the matter either filterable or non-filterable that remains as residue upon evaporation and subsequent drying at a defined temperature. Different forms of solids are defined on the basis of method applied for their determination. Solids may affect water or waste water quality adversely in number of ways. Water with high dissolved solids may induce an unfavorable physiological reaction in the transient consumer and generally affect the palatability.

Highly mineralized waters are unsuitable for many industrial applications. Highly suspended solids in waters may be aesthetically unsatisfactory for bathing or other domestic use. Determinations of solids are important to decide upon the various unit operations of water and waste-water treatment. This is more useful in the case of effluent treatment processes.

All solids are measured gravimetrically.

Alternative methods:

(a) Settle able Solids are also measured volumetric method.
(b) Total dissolved solids by specific conductance method.

After the evaporation and subsequent drying in an oven at specific temperature 103-105°C (or) 180°C,

The residue left of known samples is considered as total solids. Total solids include total suspended solids and total dissolved solids. Loss in Weight on ignitin of the sample at 500+5°C, in which organic matter is converted into Carbon dioxide & water (CO2 and H2O). Where as at controlled temperature to present decomposition and vitalization of inorganic matter as much as consistent with complete oxidation of organic matters are volatile solids.

PRINCIPLE: Total solids are determined as the residue left after evaporation and drying of the unfiltered water sample.

PROCEDURE:

1. A clean porcelain dish (china dish) is ignited in a muffle furnace and after partial cooling in the air; it is cooled in a desiccators and weighed. This gives about the empty weight of the cleaned China dish.

2. Latter, 100ml of well mixed water sample is placed in the China dish and the sample is evaporated at 100°C on water bath and later followed by drying in a hot air oven at 103°C for 1 hour.
3. Dry to a constant weight at 103°C, cool in desiccators and weighed.

OBSERVATION:

Readings are noted in the following table

S.No	Sample details	Volume of sample Taken (ml) (V)	Initial either of the China dish after Heating without Sample (mg) (A)	Final weight of The china dish With sample, After exoporation (mg) (B)	Total Solids
1	Pond water	100ml			
2	Industrial water	100ml			

CALCULATIONS

$$\text{Total Solids (mg/L)} = \frac{(B-A)\ X1000}{V}$$

A=Initial empty weight of the China dish in milligrams
B=Final weight of the China dish after evaporation of the sample in milligrams.
V=Volume of the water sample taken in milliliters.

RESULTS:

Total Solids in the pond water sample = ----------mg/L
Total solids in the industrial waste sample = ---------- mg/L

High concentration of dissolved solids about 3000mg/L may also produce distress in livestock. In industries, the use of water with high amount of dissolved solids may lead to scaling in the boilers, corrosion and degraded quality of the product in the case of manufacturing of products.

Importance of total solids determination:

1. Total solids determination is used to assess the suitability of potential supply of water for various uses. In case, where water softening is needed, the type of softening procedures used may be dictated by the total solids.

2. Corrosion control is frequently accomplished by the production of stabilized waters through p^H adjustment. The p^H at stabilization depends to some extent upon the total solids present as well as the alkalinity & temperature.

EXPERIMENT: 6 **DATE:**

Determination of iron using potassium dichromate: Redox indicators

Theory

As an oxidant, dichromate has some advantages over permanganate, but, as it is less powerful, its use is much more limited. It is obtainable in a state of high purity and can be used as a primary standard. Solutions of dichromate in water are stable indefinitely. The half reaction for the dichromate system is:

$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O \quad E° = 1.33\ V$

The most important application of dichromate is in its reaction with iron(II) in which it is often preferred to permanganate.

The relevant half reaction is :

$Fe^{2+} \rightarrow Fe^{3+} + e^- \quad E° = -0.77\ V$

and the total reaction is:

$Cr_2O_7^{2-} + 6\ Fe^{2+} + 14H^+ \rightarrow 2Cr^{3+} + 6\ Fe^{3+} + 7H_2O \quad E° = 0.56\ V$

Unlike permanganate, dichromate titrations require an indicator. There are three indicators that may be used for the titration of Fe^{2+} with $K_2Cr_2O_7$. These are diphenylamine, diphenylbenzidine and diphenylamine sulfonate. The colour change for all three indicators is green to violet and the standard electrode potentials are all ca 0.78 V. According to Kolthoff and Sandell, this should lie between the electrode potentials of the two reduction reactions. This not being the case, phosphoric acid is added to reduce the electrode potential for the $Fe^{3+} \rightarrow Fe^{2+}$ reaction by stabilising the ferric ion.

Method

Prepare a standard dichromate solution by dissolving an accurately weighed sample of about 0.4 g in water and make up to 100 cm3 in a volumetric flask. Into flasks or beakers weigh out accurately duplicate portions of about 0.7 g of the iron(II) solid `M'

provided. Add 30 cm3 of dil. sulfuric acid, 100 cm3 of water, 7 cm3 of 85% phosphoric acid and 5 drops of diphenylamine sulfonate indicator. Titrate with dichromate to a purple colour. Calculate the percentage of iron in the solid `M'.

EXPERIMENT: 7 **DATE:**

DISSOLVED OXYGEN (DO)

Aim: To determine the Dissolved Oxygen content in a given water sample.

Introduction:

Dissolved Oxygen is the amount of gaseous oxygen dissolved in water. Oxygen gets into the water by diffusion from the surrounding air, by aeration and through photosynthesis. Presence of adequate DO, indicate good quality of water.

Do levels in natural waters and wastewaters depends on physical, Chemical and biological activities in the water body. The solubility of DO in fresh water varies from 14.6mg/L at 0°C to about 7.0 mg/L at 35°C under normal atmospheric at any given temperature. All living organisms require Oxygen in one form or other to maintain the metabolic processes that produce energy for growth and reproduction. Dissolved oxygen is also important in precipitation and dissolution of inorganic substances in water.

Determination of DO is very important test in water and water pollution control. The following details indicate the importance of DO as very significant environmental parameter.

- DO levels are useful indicator to assess the degree of organic contamination of water sources.
- In Wastewater, DO is the factor that determines, the aerobic or anaerobic conditions in wastewater treatment.
- DO test is basis for determination of BOD, which is an important parameter to assess the potential of any wastewater.
- Aerobic biological treatment requires the presence of DO

Apparatus: BOD bottles, Pipette Burette and Conical Flask.

Methods for determination of DO

i) Winkler method (Iodometric method)
ii) Electrometric method

The Winkler Method with Azide Modification

Oxygen present in a sample rapidly oxidizes divalent manganous hydroxide to its higher valency which is precipitated as a brown hydrated oxide after athe addition of NaOH/KOH and KI.Upon acidification, manganese reverts to divalent state and liberates iodine from KI equivalent to the original DO content. The liberated iodine is titrated against $Na_2S_2O_3$ (N/80) using starch as an indicator.

Interferences:

Ferrous Iron, ferric iron, nitrite, microbial mass and high suspended solids concentration constitutes the source of interference. Modifications to reduce these interferences are described in the procedure.

Reagents

1. Manganese sulphate
2. Alkali iodide-azide reagent
3. Starch indicator
4. Standard sodium thiosulphate (0.025N)
5. Concentrated sulphuric acid.

Procedure:

1. Take the BOD bottle and collect 300 ml of water sample into it.
2. Add 2ml of manganese sulphate and 2ml of alkali iodide – azide solution to the BOD bottle. The tip of the pipette should be below the liquid level, while adding these reagents.
3. Restopper with care to exclude air bubbles and mix by repeatedly inverting the bottle 2 to 3 times.
4. If no oxygen is present, the manganese ion reacts with hydroxide ion to form white precipitate of Mn $(OH)_2$. If oxygen is present, some Mn^{++} is oxidized in Mn^{++++} and precipitates as a brown coloured manganic oxide.

   $Mn^{++} + 2(OH)^{-}s \rightarrow Mn(OH)_2$ (white)

   $Mn^{++} + 2(OH) + ½\ O_2 \rightarrow MnO_2$ (brown) $+ H_2O$

5. After shaking and allowing sufficient time for all oxygen to react, the chemical precipitates are allowed to settle leaving clear liquid within the upper portion.
6. 2 ml of concentrated sulphuric acid is added.
7. The bottle is restoppered and mixed by inverting until the suspension is completely dissolved and yellow colour is uniform throughout the bottle.
   $MnO_2 + 2I + 4H^{+} \rightarrow Mn^{++} + I_2 + 2H_2O$
8. A volume of * 203ml is taken into the conical flask and titrated with 0.025N Sodium thiosulphate solution until yellow coloured iodine turns to a pale straw colour.
9. Since it is impossible to accurately titrate the sample to a colourless liquid, 1 to 2 ml of starch solution is added.
10. Continue titration to the first disappearance of the blue colour.

Observations:

Sample Details	Volume of Sample Taken (ml)	Initial burette reading (ml)	Final burette reading (ml)	Ml Of $Na_2 S_2O_3$ Solution used	D.O.in (mg/L)

Calculations:

1ml of 0.025N $Na_2S_2O_3$ is equivalent to 0.2mg of O_2 since the volume of the sample is 200ml

1 ml of Sodium thiosulphate is equivalent to

(0.2 x 1000 / 200)mg/I = 1 mg/L

$$\text{D.O.in mg / L} = \frac{\text{ml of } Na_2S_2O_3 \text{ used X } 200}{\text{Vol of Sample}}$$

Results:

Significance:

Oxygen is poorly soluble in water. Its solubility is about 14.6 mg/I for pure water at 0°C under normal atmospheric pressure and it drops to 7 mg/I at 35°C. Aerobic bacteria thrive when free oxygen is available in plenty. Aerobic conditions do prevail when dufficient D.O. is available within water. End products are stable and are not foul smelling.

While a minimum D.O. of 4 to 5 mg/I is desirable for the survival of aquatic life, hjighervalues of D.O. may cause corrosion of iron and steel.

Algae growth in water may release oxygen during its photosyntheiss and D.O. may even shoot up to 30 mg/I.

Drinking water should be rich in D.O. for good taste.

Applications:

1. It is necessary to know D.O. levels to assess quality of raw water and to keep a check on stream pollution.
2. D.O. test is the basis for BOD test which is an important parameter to evaluate organic pollution potential of a waste.
3. D.O. test is necessary for all aerobic biological waste water treatment processes to control the rate of aeration.
4. 4. Oxygen is an important factor in the corrosion of iron and steel. D.O. test is used to control oxygen in boiler feed waters.
5. D.O. test is used to evaluate the pollution strength of domestic and industrial wastes.

EXPERIMENT: 8 **DATE:**

Nitrates

Aim: To determine nitrates of the given water sample.

Introduction

Nitrate is a well-known contaminant of ground and stream water. It is an important environmental and human health analyte, and thus its detection and quantification are considered to be essential. An excellent review on the detection and determination has been reported by Moorcroft et al . Most of the recent work concerning nitrate determination has embraced the classical reagents. Several reported spectro photometric methods involve the use of common reactions, such as a reduction reaction followed by diazotization,nitration reactions or others . Other methods involve the use of ion chromatography and specific ion electrodes . The well-known spectro photometric methods for the determination of nitrate are based on the nitration of phenolic compounds, chromophoric acids, 2,4-xylenol , 2,6-xylenol, 3,4-xylenol ,phenoldisulfonic acid , brucine and phenol 4-aminoazobenzene . Some sensitive spectrophotometric methods for determine nitrate utilize extractable ion associates of the nitrate ion with basic dyes, like crystal violet and nile blue.In this work, a simple and rapid method has been proposed for the determination of nitrite using methylanthranilate as a coupling agent. Sulfanilic acid was diazotized in acidic medium and coupled with methylanthranilate to give a colored dye having absorption maximum at 493 nm. Determination of nitrate is based on the reduction of nitrate to nitrite in the presence of Zn/NaCl. The produced nitrite is subsequently diazotized with sulfanilic acid and then coupled with methylanthranilate to form an azo dye and was measured at 493 nm.The developed method has been successfully applied to the determination of nitrate in different samples.

Apparatus: UV-Visible spectrophotometer with 1 cm matching quartz cells were used for the absorbance measurements

Reagents:

All chemicals used were of analytical reagent grade, and doubly distilled water was used in the preparation of all solutions in the experiments. Nitrate solution (1000 □gmL^{-1}) was prepared by dissolving 0.7220 g potassium nitrate in water and diluting to 100 mL. Working standard solutions were prepared by appropriate dilution. Sulfanilic acid (0.5 g in 100 mL water)

and methyl anthranilate (0.5 mL in 100 mL of alcohol) were used. The following reagents were prepared by dissolving appropriate amounts of reagents in water: 2 mol L^{-1} HCl & 2 mol L^{-1} NaOH.

Procedure:

Pipetted out 10 mL of nitrate stock solution to a beaker, added 5 mL of Conc. HCl and 2 mL of Zn/NaCl granular mixture0, and was allowed to stand for 30 minutes. With occasionally stirring to form nitrite, then the solution was filtered to 100 mL standard flask using Whatman No 41 filter paper and diluted up to the mark. Aliquots of stock solution containing 0.26-10.7 μgmL^{-1} of reduced nitrate were transferred in to series of 10 mL standard flask. Added 1 mL of 0.5% sulfanilic acid and 1 mL of 2 mol L^{-1} HCl solution, shaken thoroughly for 5 minutes for the diazotization reaction to go to completion. Then, 1 mL of 0.5% methyl anthranilate and 2 mL of 2 mol L^{-1} sodium hydroxide solution were added to form an azo dye and the contents were diluted to 10 mL with water. After dilution to 10 mL with water, the absorbance of the red colored dye was measured at 493 nm against the corresponding reagent blank.

Observations:

Results:

EXPERIMENT: 9 **DATE:**

PHOSPHATE DETERMINATION BY ASCORBIC ACID METHOD (SPECTROPHOTOMETRY)

INTRODUCTION: Phosphorous occurs in waters and wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro, meta, and polyphosphates) and organically bound phosphates. They occur in solution, in particles, or in the bodies of aquatic organisms.

Domestic wastewater is relatively rich in phosphorous compounds. Most heavy duty synthetic detergent formulations designed for household market contain large amounts of polyphosphates. The organisms involved in the biological processes of wastewater treatment all require phosphorous for reproduction and synthesis of new cell tissue. Domestic wastewater contains amounts of phosphorous far in excess of the amount needed to stabilize the limited quantity of organic matter present. Many industrial wastes however do not contain sufficient quantities of phosphorous for optimum growth of the organisms used in treatment. In such cases, the deficiencies may be supplied by the addition of inorganic phosphates.

Phosphate compounds are widely used in steam power plants to control scaling in boilers.

OBJECTIVE:

The objective of the experiment is to determine PO_4 (phosphate) in water and wastewater.

REAGENTS:

Stock Solution:

This solution is only needed to make standard solution.

Dissolve 219.5 mg of anhydrous KH_2PO_4 and dilute to 1,000 ml. This solution will be 50 ug P/ ml.

Standard Solution:

Dilute 25 ml of stock solution to 500 ml. This will be 2.5 ug P/ml.

The working standard is 2.5 ug/ml (2500 ppb) of phosphorus in phosphate (PO4-P). Use it to make a series of standards.

Standard Solutions

Volume (ul) used of the working standard, diluted to 25 ml.	Standard Conc. (ppb PO4-P)
ZERO	BLANK
100	10.0
200	20.0
400	40.0
600	60.0
800	80.0

5N Sulufuric Acid:

Dilute 70 ml of conc. sulfuric acid to 500 ml.

Potassium antimonyl tartarate - $K(SbO)C_4H_4O_6.1/2\ H_2O$:

The bottle of the solid salt may be labeled as: Potassium antimoy(III) oxitartarate, or Antimony Potassium Tartarate.

Dissolve 1.3715 g in 400 ml H_2O, Dilute to 500 ml. Store in glass bottle.

4% Ammonium Molybdate - $(NH_4)_6 Mo_7O_{24}.4H_2O$:

Dissolve 20 g in 500 ml. Stir for sometime (HARD TO DISSOLVE !!)

Store in glass stoppered bottle.

Ascorbic Acid:

Dissolve 1.76 g in 100 ml H_2O.

This solution is stable for ONE WEEK ONLY at 4 degrees C.

Combined Reagent:

For 100 ml of the combined reagent add the following, IN ORDER:

- 50 ml of 5N sulfuric acid.
- 5 ml Potassium antimonyl tartarate. STIRR THOROUGHLY
- 15 ml Ammonium Molybdate. STIRR THOROUGHLY
- 30 ml Ascorbic acid. STIRR THOROUGHLY
- Let the reagent cool down to room temperature.

This combined reagent is stable for 4 HOURS ONLY !!!

PROCEDURE:

- Take 25 ml sample in a 50-ml graduated tube
- Add 4 ml combined reagent.
- Cover the tube with parafilm and shake well.
- Wait for blue color to develop. It needs 10-30 minutes time.
- Measure absorbance on the Spectronic-21 at the wavelength 880 nm.

EXPERIMENT: 10 **DATE:**

BIOCHEMICAL OXYGEN DEMAND

Aim: To determine Biochemical oxygen Demand (BOD) exerted by the given waste water sample.

Introduction:

The biochemical oxygen demand (BOD) is defined as the amount oxygen required for biological oxidation of organic matter (biodegradable) by microorganisms in the presence oxygen of a given sample under controlled conditions of temperature and incubation period.

The BOD test used to determine

i) Pollution load of wastewaters (due to biodegradable organic matter)
ii) The level of organic pollution in water bodies.
iii) To assess the performance of wastewater treatment plants

Principle:

The test measures the amount of oxygen utilized during the incubation period for biodegradation of organic material (Carbonaceous demand) and oxygen used to oxidize inorganic material such as sulphides and ferrous iron by determining the initial and final DO. It also measures to oxidize reduced forms of nitrogen (nitrogen demand) unless their oxidation is prevented by the addition of inhibitor.

It is necessary to provide ideal conditions like nutrient supply, pH (6.5-7.5), absence of microbial growth inhibiting substances and temperature. The low solubility of oxygen in water necessitates strong waste to be diluted to ensure that the demand does increase the available oxygen. A mixed group of microorganisms should be present in the sample. Alternatively acclimatized seed must be added to the sample. Generally, the temperature is controlled at 20°C and test conducted for an incubation of 5 days, as 65-70% of the waste is oxidized during this period. The test can be performed at any other temperature with corresponding decrease in incubation period. For example BOD5 at 20°C is equivalent to BOD3, 27°C) while reporting the results, incubation period in days and temperature in °C needs to be specified.

Interferences:

Since DO estimation is the basis of BOD test, sources of interference in BOD tests are same as in the DO test. Lack of nutrients in dilution water and acclimatized seed organisms and presence heavy metals or other toxic materials are other sources of interference in this test.

Apparatus:

Incubator

Laboratory glassware including 300mL BOD bottles

Reagents:

1. Phosphate buffer: Dissolve 8.5g KH_2PO_4, 21.75g K_2HPO_4, 33.4g, $NaHPO_4$, $7H_2o$ and 1.7g NH_4Cl in distilled water and dilute to 1000mL. The pH should be 7.2 without further adjustment. Discard reagent if there is any sign of biological growth.
2. Magnesium sulfate: Dissolve 22.5 g $MgSO_4$, $7H_2O$ in 1000mL of distilled water.
3. Calcium Chloride: Dissolve 27.5g anhydrous $CaCl_2$ in 1000 mL of distilled water.
4. Ferric Chloride: Dissolve 0.25g $FeCl_3$. $6H_2O$ in 1000mL distilled water.
5. Sodium sulfite solution: Dissolve 1.575g Na_2SO_3 in distilled water and dilute to 1000 mL. Solution should be prepared freshly.
6. Acid & Alkali solution 1N: Prepare 1N H_2SO_4 & 1N NaOH for neutralization of caustic or acidic samples.
7. Nitrification inhibitor: 2-chloro-6-Itrichloromethyl)pyridine (Nitrification inhibitor 2570-24(2.2%TCMP), Hach Co. equivalent)
8. Glucose-glutamic acid solution: Dry reagent grade glucose and glutamic acid at 103°C for 1 hr. Dissolve 150mg glucose and 150mg glutamic acid in distilled water and dilute to 1000mL. Prepare fresh before use.
   Additionally all reagents listed in DO estimation are required.

Procedure:

A. Preparation of dilution water

   1. The source of dilution water may be distilled water, tap or receiving – stream water free from biodegradable organics and bioinhibitory substances such as chlorine or heavy metals.
   2. Aerate the required volume of dilution water in a suitable container by passing compressed air for sufficient time to attain DO saturation at room temperature. Before use stabiles the water at 20°C.
   3. Add 1 mL each of phosphate buffer, magnesium sulphate, calcium chloride, and ferric chlorfide solution for each litre of dilution water. Mix well. Quality of dilution water may be checked by incubating the sample at experimental conditions.i.e., at 20°C for 5days. Determine initial and final DO as mentioned in the procedure for DO determination. DO uptake in 5 days at 20°C should not be more than 0.2mg/L and preferably not more than 0.1mg/L.
   4. In case of the wastes which are not expected to have sufficient microbial population, add seed to the dilution water. Preferred seed is effluent from the biological treatment system. Where this is not available, supernatant from domestic wastewater (Domestic

sewage) settled at room temperature for at least 1hr but not more than 36 hours considered sufficient in the proportion 1-2 mL/L of dilution water. Adopted microbial population can be obtained from the receiving water body preferably 3-8 km below the point of discharge. Alternatively develop adopted seed in the laboratory.

5. Determine BOD of the seed at par with any other sample. This is seed control. From the value of the seed control and knowledge of the seeding material dilution (in the dilution water) determine the seed uptake. The DO uptake of seeded dilution water should be between 0.6mg/L and 1.0mg/I.

B. <u>Sample treatment</u>

1. Neutralize the sample of pH around 7, if it is highly acidic or alkaline.
2. The sample should be free from residual chlorine. If it contains residual chlorine remove it by the addition of $Na_2S_2O_3$, as follows:
   Take 50mL of the sample and acidify with the addition of 10mL 1+1 acetic acid. Add about 1g KI. Titrate with 0.025 N, $Na_2S_2O_3$, and using starch indicator. Calculate the volume of $Na_2S_2O_3$ required per mL of the sample and accordingly add to the sample to be tested for BOD.
3. Certain industrial waste, e.g. plating waste, contains toxic metals. Such samples often required special study and treatment.
4. Bring samples to 20° ± 1°C before making dilution
5. If nitrification is desired add 3 mg, 2-chloro-6-Itrichloro methyl) pyridine (TCMP) to each 300mL bottle before capping or add sufficient amounts to the dilution water to make a final concentration of 30.0mg/L. Note the use of nitrogen inhibition in reporting results.
6. Samples having a high DO content, i.e., DO 9mg/L or above due to algal growth or some other reason, reduce the DO content to saturation at 20°C by agitating or aerating with clean, filtered compressed air.

C. <u>Dilution of sample</u>

1. Dilutions that results in a residual DO of at least 1mg/L and DO uptake of at least 2 mg/L after 5 days of incubation, produce reliable results. Make several dilutions of the pretreated sample so as to obtain about 50% depletion of DO in dilution water or DO uptake in this range. Prepare dilution as follows;
   Siphon out half the required volume of seeded dilution water in a graduated cylinder or volumetric flask without entraining air. Add the desired quantity of carefully mixed sample and dilute to the appropriate volume by siphoning dilution water. Mix well with plunger type mixing rod to avoid entraining air.
   0.1% to 1.0% strong trade sewage
   1% to 5% raw of settled sewage

5% to 25% treated wastewater
25% to 100% river water

2. Siphon the dilution as in 1 in three labeled bottles and stopper immediately.
3. Keep one bottle for determination of the initial DO and incubate 2 bottles at 20°C for 5 days. See that the bottles have a water seal.
4. Prepare a blank in triplicate by siphoning plain dilution water (without seed) to measure the O_2 consumption in dilution water.
5. Determine DO in the sample and in the blank on initial day and on 5th dayI9ncubation period) by Winkler method as discussed already.
6. Calculation of BOD of the sample
   i) When dilution water is not seeded
   BOD as O_2 mg/L = (D_1-D_2) 100% of dilution

   ii) When the dilution water is seeded
   BOD as O_2, mg/L = $(D_1 - D_2) - (B_1-B_2)$ 100% dilution

   iii) When seed material is added to sample or to seed control
   BOD as O_2, mg/L = $(D_1-D_2) - (Bi-Bii)$ 100% dilution

Where

D_1=DO of the diluted sample immediately after preparation, mg/L

D_2=DO of the sample after 5 days incubation period at 20°C, mg/L

B_1= DO of blank (Seeded dilution water) before incubation period, mg/L

B_2= DO of blank (Seeded dilution water)after incubation period, mg/L

F=Ratio of seed in diluted sample to seed in seed control (volume of seed in diluted sample/Volume of seed in seed control)

Bi=DO of seed control before incubation, mg/L

Bii=DO of seed control after incubation, mg/L

Observations:

Sl.No.	Volume Of sample (ml)	Dilution ratio	Initial D.O. Of sample Mg/I	Final D.O. of sample Mg/I	Initial D.O. of Blank Mg/I	Final D.O. of blank mg/I	5days BOD at 20°C (mg/I)

Calculations:

Let Initial D.O. of distilled sample = $D_\circ$

D.O. at the end of 5 days for the diluted sample = D_5

Initial D.O. of distilled water (blank) = $C_\circ$

D.O. at the end of 5 days for the distilled water (blank) = C_5

D.O. depletion of dilution water = $C_\circ - C_5$

D.O. depletion of the diluted sample = $(D_\circ - D_5)$

D.O. depletion of the diluted sample = $(D_\circ - D_5) – (C_\circ - C_5)$

BOD at 20°C of the sample

$= [(D_\circ - D_5) \times \text{Vol. of bottle / ml sample}] - (C_\circ - C_5)$

Results:

Significance:

BOD is the principle test to give an idea of the biodegradability of any sample and strength of the waste. Hence the amount of pollution can b e easily measured by it. It is the basic criteria for the control of stream pollution.

Efficiency of any treatment plant can be judged by considering influent BOD and effluent BOD and so also the organic loading on the unit.

Ordinary domestic sewage may have a BOD of 200mg/I. Any effluent to be discharged into natural bodies of water should have BOD less than 30mg/I. This is an important parameter to assess the pollution of surface water and ground waters where contamination occurs due to disposal of domestic and industrial effluents. Drinking water usually has a BOD of less than 1 mg/I and water is considered good up to 3mg/I of BOD. But, when BOD value reaches 5mg/I, the water is doubtful in purity.

Applications:

1. To determine strength of domestic and industrial sewage.
2. The determination of BOD is used in studies to measure the self purification capacity of streams and serves regulatory authorities as a means of checking the quality of effluents discharged to such waters.
3. BOD of wastes is useful in the design of treatment facilities.
4. It is a factor in the choice of treatment method and is used to determine the size of certain units, particularly trickling filters and activated sludge units.
5. It is used to evaluate the efficiency of various treatment units.
6. It is useful to estimate population equivalent of any industrial wastes which is used for collecting cess from industries for purification of industrial wastes in municipal sewage treatment plant.
7. It is the only parameter to give an idea of the biodegradability of any sample and self purification capacity of rivers and streams.

EXPERIMENT: 11 **DATE:**

Chemical oxygen demand

Aim: To determine the Chemical Oxygen Demand (COD) of a given sample.

Introduction:

Chemical Oxygen Demand (COD) test determines the oxygen equivqlent of organic matter that is susceptible to oxidation with strong chemical oxidant. The rest can can be empirically related to BOD, organic matter or organic carbon for samples from specific source taking into account its limitations. The test is useful in studying performance evaluation of wastewater treatment plants and monitoring relatively polluted water bodies.

The intrinsic limitation of the test lies in its inability to differentiate between the biologically oxidizable and biologically inert matter and to find out the system rate constant of aerobic biological stabilization.

COD determination has an advantage over BOD determination. COD results can be obtained in 3-4 hours as compared to 3-5 days for BOD determination. Further, the test is relatively easy, precise, and is unaffected by interferences as in the BOD test.

The open reflux method which is suitable for wide range of wastes where a large sample size is preferred is discussed below. The close reflux(titrimetric & colorimetric) methods using metallic salt reagents are more economical but require homogenization of samples to obtain reproducible results. Those methods are not discussed further, since ampoules and culture tubes with permeated reagents are available commercially. Moreover, for performing the results, instructions furnished by the manufacturer are to be followed.

Open Reflux Method:

Principle:

The organic matter gets oxidized completely by $K_2Cr_2O_7$ and silver sulphate catalyst in the presence of concentrated sulphuric acid to produce CO_2 and H_2O. The excess $K_2Cr_2O_7$ remaining after the reaction is titrated with $Fe(NH_4)_2(SO_4)_2$. The dichromate consumed gives the O_2 required for oxidation of the organic matter. The chemical reactions involved in the method are given below:

$$2\ K_2Cr_2O_7 + 8\ H_2SO_4 \longrightarrow 2\ K_2Cr_2O_4 + 2Cr_2(SO_4)_3 + 8H_2O + O_2$$

$$C6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

$$Cr_2O_7 + 6Fe + 14H \longrightarrow 6Fe + 2Cr + 7H_2O$$

Interferences:

Fatty acids , straight chain aliphatic compounds, aromatic hydtrocarbons, chlorides, nitrate and iron interfere in the system.

The interference caused by the addition of mercuric sulphate to the sample prior to the addition of other reagents. About 0.4g of mercuric sulphate is adequate to complex 40 mg chloride ions in the

form of poorly ionized soluble mercuric chloride complex. Addition of Ag_2SO to concentrated H_2SO_4 as a catalyst stimulates the oxidation of straight chain aliphatic and aromatic compounds. Nitrite nitrogen exerts a COD of 1.14 mg/mg NO_2-N. Sulphanic acid at the rate of 10mg/mg NO_2-N may added to $K_2Cr_2O_7$ solution to avoid interference caused by NO_2-N.

For complete oxidation of organic matter, it is necessary to take volumes of sulphuric acid and sample plus potassium dichromate in 3.2:1 ratio. However, to maintain the ratio, the volumes of oxidant/sample and strength of oxidation/titrant may suitably vary.

Apparatus: Reflux apparatus consisting of a flat bottom of 500ml capacity with ground glass joint and a condenser with 24/40 joint, A heating mantle or hot plate with a temperature regulator.

Reagents:

1. Standard potassium dichromate solution, 0.25N: Dissolve 12.259g $K_2Cr_2O_7$ dried at 103^0C for 24hrs in distilled water and dilute to 1000ml. Add about 120mg sulphanic acid to take care of 6 mg/L NO_2-N.
2. Sulphuric acid reagent: Add 22g Ag_2SO_4 to 9 lbs concentrated H_2SO_4 or 10g 0 1000 ml concentrated H_2SO_4 and let stand for 1 or 2 days for complete dissolution.
3. **Standard ferrous ammonium sulfate (FAS) approx. 0.1N**: Dissolve 39 g Fe $(NH_4)(SO_4)_2$. $6H_2O$ in about 400 ml distilled water. Add 20 ml concentrated H_2SO_4 and dilute to 1000 ml.

   Note: For Standardization of ferrous ammonium sulfate, dilute 10 ml standard $K_2Cr_2O_7$ to about 100 ml with distilled water, acidify by adding 10 ml H_2SO_4 and allow cooling. Titrate with the ferrous ammonium sulphate to be standardized using 2-3 drops of ferroin indicator. Calculate normality.
4. **Ferroin indicator**: dissolve 1.485 g 1, 10-phenolthalien monohydrate and 695 mg $FeSO_4.7H_2O$ in distilled water and dilute to 100 ml.
5. **Mercuric Sulphate**: $HgSO_4$ crystals, analytical grade.
6. **Potassium hydrogen phthalate (KHP) standard**: Dissolve 425 mg lightly crushed potassium hydrogen phthalate (HOOC C_6H_6COOH) in distilled water and dilute to 1000 ml. This solution has a theoretical COD of 500ug O_2/ml. This solution is stable when refrigerated up to 3 months in the absence of visible biological growth.

Procedure:

1. Place 0.4 g HgSO4 in a 500 ml reflux flask.
2. Add 20 ml sample or an aliquot of sample diluted to 20 ml with distilled water. Mix well.
3. Add clean pumic stones.
4. Add 10 ml 0.25 $NK_2Cr_2O_7$ solution and mix.
5. Add slowly 30 ml concentrated H_2SO_4 containing Ag_2SO_4 mixing thoroughly. This slow addition with swirling prevents fatty acids to escape due to high temperature.

6. Mix well. If the color turns green, either take fresh sample with lesser aliquot or add more potassium dichromate and acid.
7. Connect the flask to condenser. Mix the contents before heating. Imprope3r mixing will result in bumping and blow out of flask contents.
8. Reflux for minimum of 2hrs. Cool and then wash down the condenser with distilled water.
9. Disconnect reflux condenser and dilute the mixture to about twice its volume with distilled water. Cool to room temperature and titrate excess $K_2Cr_2O_7$ with 0.1 N FAS using 2-3 drops of ferroin indicator The sharp color change from blue green to reddish brown indicates end point. The blue –green may reappear. Use the same quantity of ferroin indicator for all titrations.
10. Reflux blank in the same manner using distilled water instead of sample.
11. Alternatively for low COD samples use 0.025 N $K_2Cr_2O_7$ and 0.025 N titrant with sample volume of 50 ml.

Calculations:

$$\text{COD as mg/L} = \frac{(a-b) \text{ X N X } 8000}{\text{Ml of sample}}$$

Where a = ml FAS used for the blank
'b= ml of FAS used for the sample
N= normality of FAS

Observations:

Sample Details	Volume of Sample (ml)	Initial Burette Reading (ml)	Final Burette Reading (ml)	Volume of H_2SO_4 Consumed (ml)	Initial Burette Reading (ml)	Final Burette Reading (ml)	Volume of H_2SO_4 Consumed (ml)
1.							
2.							
3.							
4.							
5.							

Result:

Significance:

The laboratory determinations of COD is an important experiment in measuring the organic content of a sample the COD values reflects the organic content, both biodegradable as well as non-biodegradable organic substances. The COD values help us to estimate the approximate BOD of a given sample, based on which the dilution of the given sample will be determined for BOD experiment the COD will always more than BOD. The time required for this experiment is around 3 hrs compared to 5 days required for BOD.

Applications:

The COD test used to determine

i) Pollution load of wastewaters (due to biodegradable organic matter).
ii) The level of organic pollution in water bodies
iii) To assess the performance of wastewater treatment plants.

EXPERIMENT: 12 **DATE:**

JAR TEST – DETERMINATION OF ALUM DOSE

Aim: To determine the Optimum coagulant dosage required for water treatment.

Introduction:

Chemical Coagulation, flocculation and sedimentation together reduce suspended and colloidal solids, phosphorous, fluorides, organic matter and certain toxicants. Alum, ferrous and ferric salts, when used for clarification results in producing better quality water than by using plain sedimentation. The optimum dose of these coagulants cannot be theoretically calculated and therefore laboratory tests needs to be carried out using Jar Test apparatus. This enables the investigations of such interrelated factors like pH, colour, turbidity, mineral matter, temperature, time of flocculation and the degree of agitation, which all control the coagulation and flocculation.

Principle metal salts hydrolyze in presence of the natural alkalinity to form metal hydroxides. The multivalent cation can reduce the Zeta-potential while the metal hydroxides are good absorbents and hence remove the suspended particles by enmeshing them.

Principle:

Metal salts hydrolyse in presence of the natural alkalinity to form metal hydroxides. The divalent cations can reduce the zeta – potential while the metal hyroxicdes are good absorbents and hence remove the suspended particles by enmeshing them.

Procedure:

1. Using 100ml of sample on a magnetic stirrer, add coagulant in small increments at a pH 6.0, after each addition, provide a 1 minute rapid mix followed by a 3 minute slow mix. Continue addition until a visible floc is formed. Use this dose for further experiments.
2. Take 1000ml of sample in each of six beakers as shown in the fig.
3. Adjust the pH to 4.0,5.0,6.0,7.0,8.0 and 9.0 with standard acid or alkali. Add the predetermined dose of coagulant simultaneously to all the beakers.
4. Stirr the sample (rapid mix) for 1 minute; follow this 14 minutes flocculation at slow speed.
5. Measure the turbidity of each supernatant after settling for 1 hour.
6. Plot the percent removal characteristics versus pH and select the optimum pH.
7. At this pH repeat the steps 2,4 and 5 varying the coagulant dosage.
8. Plot percent removal Vs the coagulant dosage and select the optimum dose.
9. If a polyelectrolyte is used, repeat the procedure, adding polyelectrolyte towards the end of the rapid mix.

Observations:

Sample Detail/jar No.	Dosage of Coagulant	Residual Turbidity	p^H	Alkalinity (mg/I)

Precautions:

1. Add coagulant dosage to all the beakers while stirring without much time lapse.
2. Add the dosages at the point of intimate mixing.
3. It is advisable to siphon lout the supernatant from the beakers so as to not to disturb the settled floc.
4. When using polyelectrolyte add them first before coagulant is added or advised by the supplier of the polyelectrolyte.
5. Use commercially available multiple stirrers with speed control.
6. Range of optimum p^H values should be maintained for optimum utilization of the coagulant.

Result:

Optimum dose of Alum coagulant (mg/I) =

Significance:

Coagulation is not yet an exact science, although recent advances have been made in understanding the mechanism of the process. Therefore, selection and optimum dosages of coagulants are determined experimentally by the jar test instead of quantitatively by formula. Excess dosage of alum may contribute excess aluminum in drinking water. According to some recent investigations, aluminum is neurotoxin. Less dosages of alum do not remove turbidity in water which ultimately increase load on filters. So, the optimum dosage should be added in coagulation process to prevent the above problems. Coagulation removes not only turbidity, but also colour, micro-organisms, algae, phosphate, taste and odour producing sub-stances. The jar test must be performed on each water that is to be coagulated and must be repeated with each significant change in the quality of given water.

Applications:

1. This test is useful to identify various natural coagulants.
2. It is useful to estimate optimum dosage of coagulant required for raw waters and waste waters.

EXPERIMENT: 13 **DATE:**

Chlorine demand

Aim: To Determine the Chlorine Demand of a Given Sample of Water

Theory: The chlorine demand of a water sample will be equal to the chlorine required to be added to the water sample, as to just make the free chlorine available in the water sample, after a contact period of say 30 minutes.

Procedure

i) Place 200ml of the well mixed water samples in each of the 10No. – 250 ml bottles.
ii) Add 0.5ml of standard chlorine water to the first bottle, 1.0ml to the second, 1.5ml to the third, and so on, as to add 5ml to the tenth bottle.
iii) Shake each bottle gently and allow standing for 30minutes of contact period.
iv) Add a crystal of potassium iodide and 1 ml of concentrated hydrochloric acid to each bottle.
v) Add 1ml of starch solution to each bottle.
vi) Identify that bottle (out of 10 bottles), which contains the least blue colour (rather just appearing blue colour). The chlorine water added in this particular bottle may be recorded. Let it be xml (it will be one of the figs between 0.5ml, 1.0ml, 1.5ml …….5ml). This figure x multiplied by the sample volume (200ml) divided by 1000ml (1 litre) will give the chlorine demand of water in mg/I, as:

Chlorine demand of water in mg / L

$$= \frac{\text{ml of N/35.5 chlorine water in the identified bottle X 1000}}{\text{Ml of water sample i.e, 200 ml}}$$

= ml of chlorine water X 5

The above computed value of chlorine demand up to break point chlorination will be further increased by 0.2 to 0.3 mg/I for ensuring free chlorine to take care of subsequent contamination in the distribution system, as to compute the total chlorine demand of the water.

Results:

EXPERIMENT: 14 **DATE:**

Presumptive coli form test

It is the most often used techniques for the sanitary analysis of water. The test is used to detect coliforms(coliforms are defined as facultatively anaerobic, gram negative, non spring, rod shaped bacteria that ferment lactose with the production of acid and gas with in 24 hours of incubation at 35^{o}C that make up approximately 10% of the intestinal micro organisms of humans and after animals and have found wide spread use as indicator organisms of faecal contamination

The test is performed sequentially in three stages presumptive, confirmed and completed test. Lactose broth tubes are inoculated with different water volumes in the presumptive test. Tubes that are positive for gas production are inoculated into brilliant green lactose bile broth in the confirmed list and positive tubes are used to calculate the most probable number(MPN) of coliform in the water sample following the statistical table. The completed test involving the inoculations of EMB agar plate, and brilliant green lactose bile broth and preparation of a gram stain slide from EMB agar plate , is used to establish that confirm bacteria presence in the sample. The complete process including the confirmed and completed tests require at least 4 days.

Presumptive Coliforms Test:

The presumptive coliforms test is used to detect coliforms in a water sample. In this test lactose fermentation tubes are inoculated with different water volumes and production of acid and gas from the presumptive evidence of coliforms in the water sample. The lactose broth used in this test is selective for the isolation of coliforms. A pH indicator such as phenol red is also added to lactose broth for the detection of acid. The colour of the indicator changes to yellow with the production of acid from lactose.

Requirements:

- Water sample(100 ml)
- Lactose broth medium
- Durham tubes(15)
- 10ml double strength lactose broth tubes
- 5 ml single strength lactose broth tubes
- Sterile pipettes, one each of 10 ml, 1 ml and 0.1 capacity.
- Bunsen burner / spirit lamp
- Mechanical pipette in device
- Glass marker pencil

Procedure:

- Collect water sample from a pond or a sewage plant
- Lable 5 double strength lactose brooth tubes “10” and 5 single strength broth tubes “1” another 5 tubes “0”.
- Mix the water sample by thoroughly shaking
- Aseptially inoculate each “10” tubes with 10 ml of water sample using 10 ml sterile pipette.
- Using 1ml pipette aspetically inoculate the five tubes with 1 ml of water sample
- Using 0.1 ml pipette, aspecially inoculate the five tubes (CBIX) with 0.1 ml of water sample
- Incubate all the 15 inoculated tubes aerobically at 35 for 48 hours.

Result:

Production of acid (colour change) and gas (appearance of a bubble large enough to fill the concavity at the top of the duration tube) after 24 hours incubation indicates a positive presumptive test for coliform bacteria. If gas develops in tubes after 48 hours, incubation presumptive test is doubtful aand if there is no gas produced after 48 hours incubation , it shows negative presumptive test (i.e coliform absent) . Record the number of tubes showing the positive presumptive test . The tubes showing positive presumptive test are retained and used for confirmed test.

Confirmedd coliform test:

This test is used to confirm the presence of coliforms in water samples. Selective media EMB agar medium is used to inhibit the growth of gram positive organisms and allow the growth of gram negative organisms. EMB agar plates are prepared and innoculum from lactose broth test tubes showing gas bubbles is taken and streaked on plates. EMB plates are incubated at 37oc for 48hrs. Metallic sheen green colonies confirms the presence of *E.coli* in water samples.

Completed coliform test: Gram staining test is performed on green colonies. Red color short rods confirms the *E.coli* presence in water sample.