D.K.M. COLLEGE FOR WOMEN

(AUTONOMOUS), VELLORE

eRESOURCES

Digital Learning

E CONTENT TITLE : WASTE WATER TREATMENT

DEPARTMENT : CHEMISTRY

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Estimation of various parameters of water sample

1. **Estimation of Electrical conductivity**

2. **Estimation of pH**

3. **Estimation of TS**

4. **Estimation of TSS**

5. **Estimation of TDS**

6. Estimation of total hardness

Strength of EDTA – N1

Volume of sample	Burette reading		Volume of EDTA (ml)	Indicator
	Initial	Final		

Volume of water sample – V2 mL

Volume of EDTA - V1 mL

Strength of EDTA – N1

$$\text{Hardness, mg/L} = \frac{(\text{ml} \times \text{N}) \text{ of EDTA}}{\text{ml sample}}$$

7.Estimation of Chlorides

Strength of Silver nitrate – N1

Volume of sample	Burette reading		Volume of Silver nitrate (ml)	Indicator
	Initial	Final		

Volume of water sample – V2 mL

Volume of Silver nitrate - V1 mL

Strength of Silver nitrate – N1

$$\text{Chloride, mg/L} = \frac{\text{(ml x N) of } AgNO_3 \text{ x 1000 x 35.5}}{\text{ml sample}}$$

8.Estimation of Total Alkalinity

Strength of HCl – N1

Volume of sample	**Burette reading**		**Volume of HCl (ml)**	**Indicator**
	Initial	**Final**		

Volume of water sample – V2 mL

Volume of HCl used with only phenolphthalein indicator - A mL

Volume of total HCl used with phenolphthalein and methyl orange indicator - B mL

Strength of HCl – N1

$$\frac{\text{(A x Normality) of HCl x 1000 x 50}}{}$$

$$\text{PA as CaCO3, mg/L} = \frac{\text{(B x Normality) of HCl x 1000 x 50}}{\text{ml of sample}}$$

$$\text{TA as CaCO3, mg/L} = \frac{\text{(B x Normality) of HCl x 1000 x 50}}{\text{ml of sample}}$$

where, A = ml of HCl used with only phenolphthalein

B = ml of total HCl used with phenolphthalein and methyl orange.

PA = phenolphthalein alkalinity

TA = total alkalinity

Concentration of carbonates, bicarbonates and hydroxyl ions can be determined from the table using data of PA and TA.

Table : Values of hydroxyl ions, carbonates and bicarbonates from the values pf phenolphthalein and total alkalinities.

Result of titration	**OH alkalinity as $CaCO_3$**	**CO_3 alkalinity as $CaCO_3$**	**HCO_3 alkalinity as $CaCO_3$**
P = 0	0	0	T
P < ½ T	0	2P	T – 2P
P = ½ T	0	2P	0
P = ½ T	2P – T	2(T – P)	0
P > ½ T	2P – T	2(T – P)	0
P = T	T	0	0

Where, P = Phenolphthalein alkalinity

T = Total alkalinity

9. Estimation of Acidity

Strength of NaOH – N1

Volume of sample	**Burette reading**		**Volume of NaOH (ml)**	**Indicator**
	Initial	**Final**		
1. Methyl Orange 2. Phenolphthalein			(A mL) (B mL)	

Volume of water sample – V2 mL

Volume of NaOH - A mL

Strength of NaOH – N1

Methyl orange acidity, mg/L = $\frac{\text{A x N of NaOH x 1000 x 50}}{\text{ml of sample}}$

Phenolphthalein acidity, mg/L = $\frac{\text{B x N of NaOH x 1000 x 50}}{\text{ml of sample}}$

Total acidity, mg/L = $\frac{\text{(A + B) x N of NaOH x 1000 x 50}}{\text{ml of sample}}$

where, A = volume of NaOH used with methyl orange in titrating the sample to pH 3.7

B = Volume of NaOH used with phenolphthalein in titrating the sample from pH 3.7 to pH 8.3

10. Estimation of Dissolved Oxygen

Strength of Sodium thiosulphate – N1

Volume of sample	Burette reading		Volume of thio (ml)	Indicator
	Initial	Final		

Volume of water sample – V2 mL

Volume of Thio - V1 mL

Strength of thio – N1

$$\text{Diss. Oxygen, mg/L} = \frac{(\text{ml} \times \text{N1}) \text{ of titrant} \times 8 \times 1000}{\text{V1} - \text{V}}$$

Where, V1 = volume of sample bottle after placing the stopper.

V = volume of MnSO4 and KI added.

11. Estimation of Biochemical Oxygen Demand

Strength of Sodium thiosulphate – N1

Volume of sample	Burette reading		Volume of thio (ml)	Indicator
	Initial	Final		

Volume of water sample – V2 mL

Volume of Thio - V1 mL

Strength of thio – N1

$$\text{Diss. Oxygen,(Do) mg/L} = \frac{\text{(ml x N1) of titrant x 8 x 1000}}{\text{V1 – V}}$$

Where, V1 = volume of sample bottle after placing the stopper.

V = volume of MnSO4 and KI added.

D5 - = DO after 5 days

BOD, mg/L = (D0 – D5) x dilution factor

Where, D0 = Initial DO in the sample

D5 = DO after 5 days

12. Estimation of Chemical Oxygen Demand

Strength of Ferrous Ammonium Sulphate – N1 (0.01N)

Volume of sample	**Burette reading**		**Volume of FAS (ml)**	**Indicator**
	Initial	**Final**		
1. Sample –				
2. Blank -				

Volume of water sample – V2 mL

Volume of FAS - V1 mL

Strength of FAS – N1

$$\text{COD, mg/L} = \frac{(b - a) \times N1 \text{ of Ferrous ammonium sulphate} \times 1000 \times 8}{\text{V2 ml sample}}$$

Where, a = ml of titrant with sample

B = ml of titrant with blank.

Table - Physico – chemical parameters of the water sample

S. No.	Parameters	S1	S2	S3	S4
1	Colour				
2	pH				
3	Electrical conductivity dS/cm^2				
4	Total Solids (mg/L)				
5	Total Dissolved Solids(mg/L)				
6	Total Suspended Solids (mg/L)				
7	Total hardness (mg/L)				
8	Total Alkalinity (mg/L)				
9	Total Acidity (mg/L)				
10	Total hardness (mg/L)				
11	Dissolved Oxygen (mg/L)				
12	Biochemical Oxygen Demand (mg/L)				
13	Chemical Oxygen Demand (mg/L)				

WATER

Water is one of the most abundant and important substances known to man. It is present in the air as water vapour and in the ground in the form of underground streams. Surface water in the form of oceans, rivers and lakes covers about three – quarters of the earth's crust. It is an essential constituent of all animal and vegetable matter.

Properties of Water

Properties of water seem peculiar in many respects. Of substances with low molecular mass, water has unusually high boiling point. Compared to other substances isoelectronic with water, it is many times more abundant in nature. It is further unusual in the fact that it is more dense than ice. These characteristics are due to its peculiar structure. In water and ice the water molecules are joined to each other through hydrogen bonds. The O atom is surrounded by four other O atoms (of the neighbouring water molecules) located at the corners of the regular tetrahedron and extends into three dimensions.

The existence of hydrogen bonds between water molecules is also responsible for its abnormally large specific heat and the latent heats of fusion and vopurization. When ice is melted. Water is heated or vapourized heat is required for overcoming the weak attraction between the molecules (van der Waals forces) as well as for breaking the hydrogen bonds. Hence more energy is needed for these operations in water than in any other analogous substance wherein there is no hydrogen bonding.

It is a colourless, odourless, tasteless liquid (m.p. = 273 K and b.p. = 373 K at 760 mm pressure). Due to polar character of its molecule, it has a high dielectric constant (=81). The polar character of water makes it an excellent solvent for many other polar and ionic substances.

Potable Water - Water for Domestic Supply

Municipalities have to supply potable water, i.e. water is safe to drink. Potable water should have the following characteristics.

1. It should be colourless and odourless.
2. It should have a good taste.
3. It should be clear from suspended impurities.
4. It should be free from objectionable gases like CO_2, NH_3, H_2S, etc.

5. It should be free from harmful bacteria.
6. It should not have more than 0.1 – 0.2 ppm of free chlorine.

Rivers, lakes and wells are the most common sources of water used by municipalities. The actual treatment method depends directly on the impurities present.

Screening

Screening is the process of removing floating materials like wood pieces, leaves, etc. from water. Raw water is allowed to pass through a screen, having a large number of holes, which hold back the floating matter and allow the water to pass.

Sedimentation

Sedimentation is the process of removing suspended impurities by allowing the water to sand undisturbed for 2 – 6 hours in a big tanks. Due to force of gravity most of the suspended particles settle down at the bottom and they are removed. Sedimentation removes only 70 – 75 percent of the suspended matter.

Coagulation

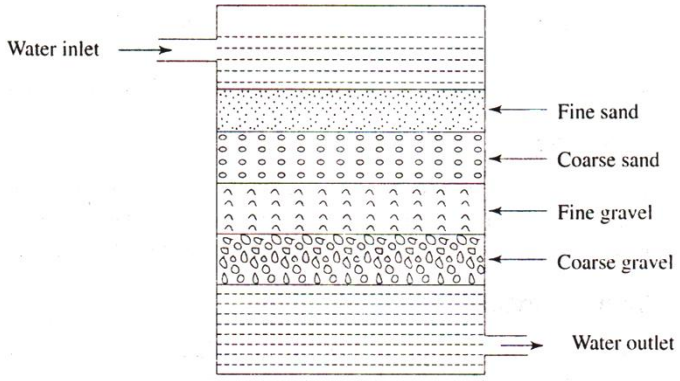
Finely divided silica, clay, etc. not settle down easily and hence cannot be removed by sedimentation. Most of these are in colloidal from and are negatively charged and hence do not coalesce because of mutual repulsion. Such impurities are removed by coagulation method. In this method, certain chemicals like alum. $Al_2(SO_4)_2$, etc. are added to water. When $Al_2(SO_4)_3$ is added to water, it hydrolyses to form a gelatinous precipitate of $Al(OH)_3$. The gelatinous precipitate of $Al(OH)_3$ entraps finely divided and colloidal impurities, settles to the bottom and can be removed easily.

Filtration

For removing bacteria, colour, taste, odour, fine suspended particles, etc. and to produce clear water, filtration is used. In this process, water is passed

through beds, of fine sand, coarse sand and other granular material. The porous material used is the filtering medium and the equipment used for filtration is known as filter, e.g., slow sand filter.

A typical slow sand filter is shown.



Slow sand filter

It consists of a tank containing thick beds of fine sand (at the top), coarse sand, fine gravel and coarse gravel (at the bottom). When the water passes through the filtering medium, it flows through the various beds slowly due to gravity. The rate of filtration slowly decreases due to the clogging of impurities in the pores of the sand bed. When the rate of flow becomes very slow, filtration is stopped and the bed is cleaned by scraping of a small layer of the sand bed (top layer) and replacing it with sand. Bacteria are also partly removed by this process.

Sterilization

The complete removal of harmful bacteria is known as sterilization. The sterilizing generally used for sterilizing water are (a) bleaching powder, (b) chlorine (c) ultraviolet rays and (d) ozone.

(a) Sterilization by chlorine or Bleaching Powder

Chlorine is the most common sterilizing agent in water treatment. Chlorine may be added in the form of bleaching powder or directly as a gas or in the form of concentrated solution in water.

When bleaching powder is added to water, HOCL which acts as a powerful germicide is produced. It is believed that HOCL reacts with bacteria and inactivates the enzymes responsible for the metabolic activities of bacteria. Since these enzymes are inactivates, all the bacteria are killed and the water is sterilized.

$CaOCl_2 + H_2O \longrightarrow Ca(OH)_2 + Cl_2$

$Cl_2 + H_2O \longrightarrow HCl + HOCl$

$HOCl + Bacteria \longrightarrow$ Bacteria are killed

(b) Sterilization by Ultraviolet Radiations

Ultraviolet radiations emanating from electric mercury vapour lamp are capable of sterilizing swimming pool water. This process is highly expensive.

(c) Sterilization by Ozone

Ozone is a powerful disinfectant and is readily absorbed by water. Ozone is highly unstable and decomposes to give nascent oxygen which is capable of destroying bacteria

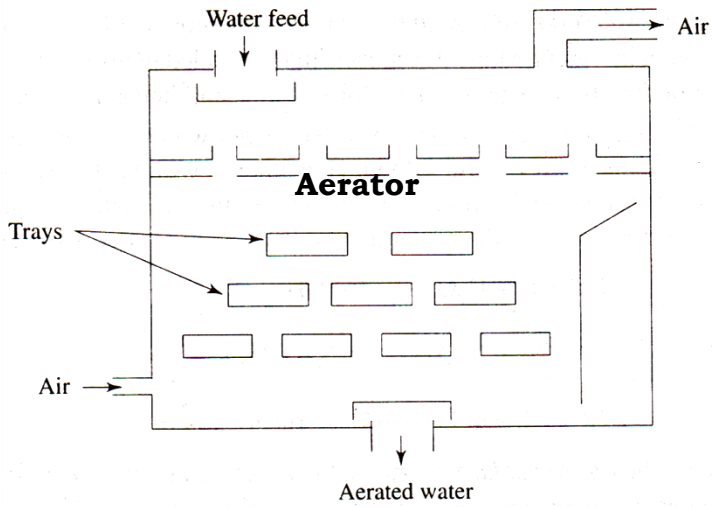
$O_3 \longrightarrow O_2 + [O]$

This process is also relatively expensive.

Aeration

The process of mixing water with air is known as aeration. By aeration (i) gases and volatile impurities (CO_2, H_2S) are mechanically reduced, and (ii) ferrous and manganous salts are removed as ferric and magnetic salts.

The mixing of water and air is done in aerators. The aerator is shown is known as forced – draught type aerator.



It consists of a tall tower containing several wooden trays. These trays are arranged in a manner that water overflowing falls exactly in the middle of the lower tray. Water is allowed from the top and is made to flow through the trays. This results in the formation of a thin film of water in – between the trays. At the same time air is blown into the aerator at the bottom. The air ascends up and mixes at various stages with water. As a result, the water collecting at the bottom after aeration is free from gases like CO_2, H_2S, etc. By aeration CO_2 contamination can be reduced from 400 ppm to 5 ppm.

Desalination

Depending upon the quantity of dissolved solids, water is graded as

(a) Fresh water – has < 1000 ppm of dissolved solids.

(b) Brackish water – has > 1000 but < 35,000 ppm of dissolved solids.

(c) Sea water – has > 35,000 ppm of dissolved solids.

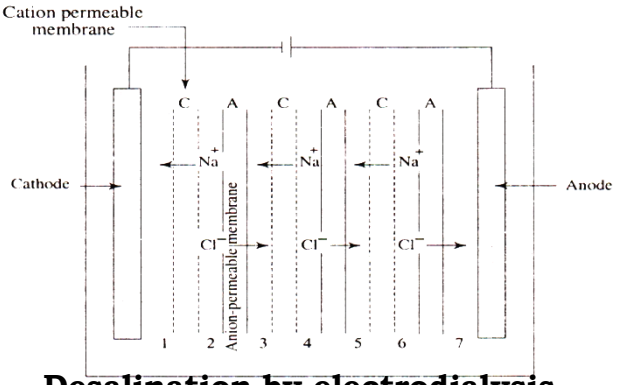
Water containing dissolved salts with a peculiar salty or brackish taste is called brackish water. It is totally unfit for drinking purposes. Sea water and brackish water can be made available as drinking water through desalination process. The need for such a method arises due to the non – availability of fresh water. Desalination is carried out either by electrodialysis or by reverse osmosis.

Electrodialysis

It is based on the fact that the ions present in saline water migrate towards oppositely charged electrodes under the influence of an applied emf. The movement of ions takes place through ion – selective membranes.

An electrodialysis cell consists of alternate cation and anion – permeable membranes. The cathode is placed near the cation – permeable membrane and the anode is placed near the anion – permeable membrane. Under the influence of an emf applied across the electrodes, the anions (Cl^-) move towards the anode and the cations (Na+) move towards the cathode. The net result is the

depletion of ions ion the even – numbered compartments and concentration of ions in the odd – numbered compartments. Now the even – numbered compartments are failed with pure water and the odd – numbered compartments are filled with concentrated brine solution. Thus, the salinity is removed from salt water.



Desalination by electrodialysis

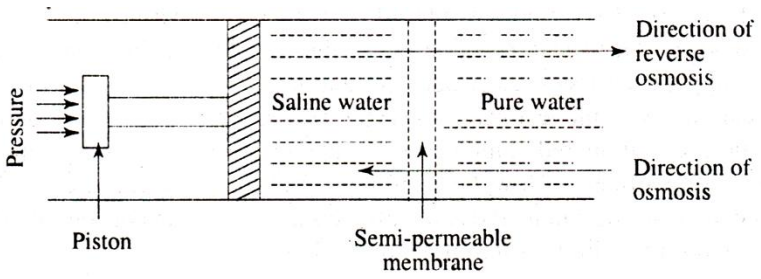
The following membrane are generally used:

Cation selective membrane – polystyrene containing sulphonic acid group.

Anion selective membrane – polystyrene containing tetra alkyl ammonium chloride group.

Reverse Osmosis

When two solutions of different concentrations are separated by semi – permeable membrane, flow of solvent takes place from a region of low concentration to high concentration until the concentration is equal on both sides. This process is called osmosis. The driving force in this phenomenon is called osmotic pressure. If a hydrostatic pressure in excess of osmotic pressure is applied on the higher concentration to lower concentration. This is the principle of reverse osmosis. Thus, in reverse osmosis method pure water is separated from its dissolved solids.



Reverse osmosis

Using this method pure water is separated from sea water. This process is also known as super – filtration. The membranes used are cellulose acetate, cellulose butyratre, etc.

WATER ANALYSIS

The water quality parameters are roughly divided into three categories. i.e., (1) Physical (2) Chemical (3) Biological.

Sampling

Selection of sampling sites, sapling methods, preservation and handling of samples for a water pollution study are discussed.

Selection of sampling sites

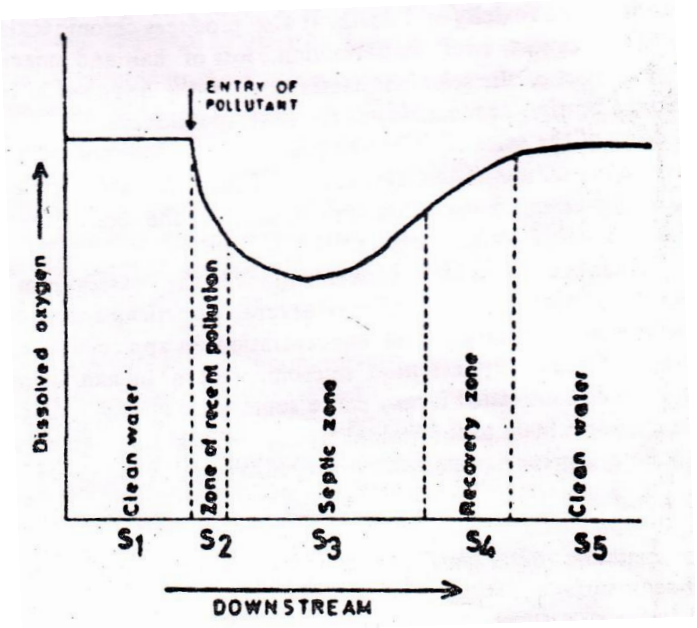
The success and failure of a water pollution survey shall depend largely upon the planning made prior to taking the samples. The plan should include not only the location of sampling sites, parameters to be analyzed, time scheduled to be followed (including the time of the day when sampling is to be done and frequency), methods of data collection but also the sample handling prior to collection.

Sampling points must be located to provide an accurate understanding of the existing water quality. The sampling on extremely remote places should

be avoided until of course, the special need arises and the facilities are adequate.

The selection of actual sampling location in the water body shall depend upon the character of the water body. In a lake or a wide river many sampling sites should be selected at various corners. If the lake is stratified, three vertical samples at one site (surface, middle and bottom) shall be required. In shallow ponds only surface and bottom samples or a sample from 0.5 to 0.7 m depth shall also suffice. In a stream which is narrow and rapidly moving the water shall also suffice. In a stream which is narrow and rapidly moving the water shall be thoroughly mixed laterally and vertically hence only one sampling point need to be selected at each location along the stream.

In an organically polluted river at least one site should be selected above the outfall of the waters and four sites should be selected downstream representing the zone of recent pollution, saprobic zone, recovery and clean water.



Collection of Samples

Samples may be collected by using a water sampler or by using glass or polyethylene bottles. For a depth sample, a sampler is often necessary. For collection of a sample from the bottom, where water is shallow (around one meter) and a depth sampling is must; the sample can be collected by lowering a closed bottle to the bottom, opening and closing it thereby had and bringing at the surface. But in this case the surface sample be collected first to avoid the disturbance caused by loose sediment.

Prior to the collection, the sample bottle must be rinsed thoroughly with the sample water if it is precleaned.

Collection of rain water

It is essential to study the characteristics of rainwater, especially in the areas of high air pollution, in order to know its likely impact on freshwaters since the contamination is ultimately to be transferred to water through precipitation.

Grab Sample

A grab sample is an ordinary sample which is taken from a particular place representing the whole water quality. This type of sample is valid only when it is certain that the water quality is not changing in a short time and effluent discharges, if any, are fairly regular.

Composite Sample

A composite sample is the one taken at various times from a locality undergoing fluctuations ad combining that to know average chemical characteristics. For example, if a stream is receiving effluent at irregular times during the day, a sample may be collected at every hour and may be combined to make a composite sample.

Collection of waste water from industries and sewerage system

It is often a part of detailed water pollution studies to study wastewater characteristics, volume generated and treatment efficiency in industrial and sewerage systems including industrial waste treatment plants and sewage treatment systems.

The first and the foremost thing for these surveys is the 'flow measurement'.

Flow Measurement

A number of methods of measuring flow in streams and wastewater carrying pipes are available. The choice of method depends largely on the affordability but the type of locale also influences selection of the method. The various devices and methods available are as follows:

Bucket Method

This method is applicable when the waste water is falling from a pipe or sewer. A bucket can be used to fill the water from this pipe, and the time taken in filling is noted by using a stopwatch flow.

$$\text{Flow (in litres / min)} = \frac{\text{Litres in bucket} \times 60}{\text{time in seconds}}$$

Surface Float Method

This is a simple approach. A float (any piece of wood, plastic, etc) is thrown on water surface. The time required for a float to travel (t), a known distance (d) is observed and the average velocity is obtained by

$$V = \frac{d}{1.2t}$$

The factor 1.2 accounts for the fact that surface velocities are normally about 1.2 times higher. If the cross – sectional area (A) is measured, the discharge Q is given by :

$$Q = VA$$

This method is useful only in shallow and small streams.

Sample Handling and Preservation

Accurate sampling should be followed by correct handling and preservation of the sample to obtain the reliable results. A few hints are given here :

1. Immediately after collection, clearly label each sample bottle with water proof ink, and record the relevant details for each sample.
2. Temperature of water should be immediately recorded.
3. The samples for chemical, biological, and bacteriological analyses should preferably be collected separately.
4. The sample should be taken to the laboratory as early as possible; it should be protected from direct sunlight during transportation.

Since most of the parameters change with time, it is imperative to preserve them in a suitable preservative prior to analyses. Dissolved Oxygen, Free CO_2, Alkalinity and pH quickly change with time hence their estimation is to be carried out in the field only.

Table : Preservation techniques of water samples for chemical analyses.

Parameter	**Recommended sample volume (ml)**	**Type of container**	**Preservation**	**Allowable holding time**
Acidity	100	P,G	Refrigerate -4^{o}C	24hr
Alkalinity	100	P,G	Refrigerate -4^{o}C	24hr
BOD	1000	P,G	Refrigerate -4^{o}C	6hr
COD	50	P,G	H_2SO_4 to pH>2	7 days
Chlorine, residual	500	P,G	Analyze immediately	-
Chloride	50	P,G	Not required	7 days

Colour	50	P,G	Refrigerate -4^{o}C	24hr
Cynaide	500	P,G	Refrigerate -4^{o}C; NaOH to pH 12	24hr
Dissolved Oxygen :				
Probe	300	G	Determine on site	-
Winkler	300	G	Fix on site	6 hr
Hardness	100	P,G	Refrigerate -4^{o}C	7 days
Metals	200	P,G	HNO_3 to pH<2	6 months
Ammonia	500	P,G	Refrigerate -4^{o}C; H_2SO_4 to pH<2	24hr
Kjeldahl – N	500	P,G	Refrigerate -4^{o}C; H_2SO_4 to pH<2	7 days
Nitrate	100	P,G	Refrigerate -4^{o}C; H_2SO_4 to pH<2	24 hr
Nitrite	100	P,G	Refrigerate -4^{o}C	24 hr
Oil and Grease	1000	G	Add HCl to pH<2	24hr
Organic Carbon	50	P,G	Refrigerate -4^{o}C; H_2SO_4 to pH<2	24 hr
pH	100	P,G	Determine on site	-
Phenol	500	G	Refrigerate -4^{o}C 1.0g $CaSO_4$/<2	24hr
Phosphate	50	P,G	Refrigerate -4^{o}C	24hr
Specific Conductance	100	P,G	Refrigerate -4^{o}C	24hr
Solids (total dissolved)	100	P,G	Refrigerate -4^{o}C	7 days
Sulphate	50	P,G	Refrigerate -4^{o}C	7 days
Sulphide	100	P,G	Analyze immediately	-
Temperature	1000	P,G	Determine on site	-

Turbdity	100	P,G	Refrigerate -4^{o}C	No holding
Radioactivity	1000	P,G	Not required	24 hr

Water and Sediment Sampling Equipments

The sampling of water from the bank of a stream shallow areas in a pond can be easily carried out using a wide mouthed polyethylene bottle but a sample has to be used if the studies are to be undertaken at various strata of water. Such studies are often essential in freshwater ecology in view of chemical and biological stratification in lacustrine waters and sometimes even in a river.

Analysis of water samples

Temperature

The parameter of temperature is basically important for its effects on the chemistry and biological reactions in the organisms in water. A rise in temperature of the water leads to the speeding up of the chemical reactions in water, reduces the solubility of gases and amplifies the tastes and odours. Water in the temperature range of 7^{o}C to 11^{o}C has a pleasant taste and is refreshing. At higher temperature with less dissolved gases, the water becomes tasteless and even does not quench the thirst. At elevated temperatures metabolic activity of the organisms increases, requiring more oxygen but at the same time the solubility of oxygen decreases, thus accentuating the stress.

Temperature is also very important in the determination of various other parameters such as pH. Conductivity, saturation level of gases and various forms of alkalinity, etc. Data on temperature is also required by the industries in heat transmission calculations, cooling towers and process use.

Colour

Even pure water is not colorless. It has got a pale green – blue tint in large volumes. Colour in natural waters may occur due to the presence of humic acid, fulvic acids, metallic ions such as iron and manganese, suspended

matter, phytoplankton, weeds and industrial wastes, etc. Colour due to organic acids may not be harmful as such, but highly coloured waters are objected on aesthetic grounds. Coloured waters may not be accepted for certain uses in the industries. Several industries use artifical colours (e.g., textile) which come out in their wastes.

Taste and Smell

The taste and odours are present mainly due to dissolved impurities often organic in nature. They are supposed to be 'chemical senses' as they depend on the actual contact with the receptor organ.

The odours may be natural origin, caused by living and decaying aquatic organisms, and accumulation of gases like ammonia and hydrogen sulphide, etc. Many algae also impart taste and odours to water. Odours of any artificial origin are due to the discharge of industrial wastes which include many chemicals imparting odour and tastes. Sometimes, reagents added to water supply systems may also produce odour and tastes. The chlorine added for disinfectin of water reacts sometimes with organic matter to form chlorophenols which possess a very high sensitivity for taste and odours.

The objectionable tastes and odours are sometimes rejected on the ground of aesthetic value. Some organic substances imparting taste and odours may also be toxic. The tastes and odours in the water are also not suitable in food, pharmaceuticals and beverage industries.

Turbidity

Turbidity in water is caused by the substances not present in the form of true solution. True solutions have a particle size of less than 10^{-9} m. Any substance having more than the size will produce a turbidity. Turbidity cf water is actually the expression of optical property (Tundall effect) in which the light is scattered by the particles present in the water.

Turbidity in natural waters is caused by clay, slit, organic matter, phytoplankton and other microscopic organisms. Turbidity determinations do not correlate with the actual amount of suspended matter as the scattering of light is highly dependent upon the size, shape and refractive index of the particles.

Turbidity makes the water unfit for domestic purposes, food and beverage industries, and many other industrial uses. Reconjuction of turbidity is an important objective in removal of the turbidity on coagulation filtration, etc., in drinking water plants. A reduction in turbidity is associated with a reduction in suspended matter and microbial growth. Turbidity in natural waters restricts light penetration for photosynthesis.

Conductivity

Conductivity is the measure of capacity of a substance or solution to conduct electric current. Conductivity is reciprocal of the resistances. Conductivity if measured of a cube with each side of 1 cm at 25^{o}C is called *specific conductance*. As most of the salts in the water are present in the ionic forms, capable of conducting current, therefore; conductivity is a good and rapid measure of the total dissolved solids. As the dissolved solids in the highly mineralized waters are usually more than 65% of the conductivity, the value of dissolved solids, as a general rule, can be obtained by multiplying it by a factor of 0.65. However, it is difficult to get accurate values by this calculation, as capacity to conduct the current depends on number and kinds of ions present, their charge, and freedom of ions to act as conductors. Solids should not be estimated from the conductivity value, if it exceeds 50 mmho (50, 000 μmho).

The conductivity of distilled water ranges between 1 to 5 μ mho but the presence of salts and contamination with waste waters increase the

conductivity of the water. Consequently, a sudden rise in conductivity in the water will indicate addition of some pollutants to it.

The conductivity is generally reported in mmho or μmho. The recent unit of conductivity has been named as Siemens (S) instead of mho. Conductivity is highly dependent upon temperature and therefore is reported normally at 25^{o}C to maintain the comparability of the data from various sources. It has got no health significance as such. Conductivity, however, is an important criterion in determining the suitability of water and waste water for irrigation. Waters having conductivity more than 20 mmho have not been found suitable for irrigation.

pH (Potentia hydrogenii)

pH is the measure of the intensity of acidity or alkalinity and measure the concentration of hydrogen ions in water. It does not measure total acidity or alkalinity. In fact, the normal acidity or alkalinity depends upon excess of H^+ or OH^- ions over the other, and measured in normality or gram equivalents of acid or alkali. If free H^+ or OH^- ions over the other, and measured in normality or gram equivalents of acid or alkali. If free H^+ are more than OH^- ions, the water shall be acidic, or alkaline the other way round.

Most natural waters are generally alkaline due to presence of sufficient quantities of carbonates. pH of water gets drastically changed with time due to the exposure to air, biological activity and temperature changes. Significant changes in pH occur due to disposal of industrial waters, acid mine drainage, etc. In natural waters, pH also changes diurnally and seasonally due to variation in photosynthetic activity which increase the pH due to consumption of CO_2 in the process.

Most chemical and biological reactions occur a narrow range of pH. Determination of pH is one of the important objectives in treatment of the wastes. In anaerobic treatment if the pH goes below 5 due to excess accumulation of acids, the process is severely affected adversely. Shifting of pH beyond 5 to 10 upsets the aerobic treatment of the wastes. In these circumstances the pH can be adjusted by addition of suitable acid or alkali to optimize the treatment of the wastes.

Total solids (TS)

Total solids are determined as the residue left after evaporation of the unfiltered sample.

Procedure

1. Take an evaporating dish (made up of silica, porcelain or platinum) of at least 100ml capacity. Ignite at 550 ±50^{o}C in a muffle furnance for about an hour, cool in a desiccator and weigh.
2. Evaporate 100ml of unfiltered sample (or more in case the solids are less than 250 Mg/L) in a evaporating dish on a water bath or a hot plate having temperature not more than 98^{o}C.
3. Heat the residue at 103 – 105^{o}C in an oven for one hour and take the final weight after cooling in a desiccator.

Calculation

$$\text{Total Solids mg/L} = \frac{A - B \times 1000 \times 1000}{V}$$

where, A = Final weight of the dish in g

B = Initial weight of the dish in g

V = Volume of sample taken in ml

Total Dissolved Solids (TDS)

Total dissolved solids are determined as the residue left after evaporation of the filtered sample.

Procedure

1. Take an evaporating dish (see Total Solids determination) and ignite it at 550 ± 50°C in a muffle furnance for about an hour, cool in a desiccator and weigh.
2. Filter the sample through glass fibre filter paper applying the suction.
3. Evaporate 100ml of this filtered sample (or more in case the solids are less than 25 mg/L) in the pre weighed evaporating dish on a water bath or a bot plate having temperature not more than 98°C.
4. Heat the residue at 103 – 105°C in an oven for one hour and take the final weight after cooling in a desiccator.

Calculation

$$\text{TDS, mg/L} = \frac{A - B \times 1000 \times 1000}{V}$$

where, A = Final weight of the dish in g

B = Initial weight of the dish in g

V = Volume of sample taken in ml

Total Suspended solids (TSS)

Determine total suspended solids as the difference between the total solids and total dissolved solids. TSS = TS – TDS.

Total Alkalinity, Carbonates and Bicarbonates

Total alkalinity is the measure of the capacity of the water to neutralize a strong acid. The alkalinity in the waters is generally imparted by the salts of

carbonates, bicarbonates, phosphates, nitrates, borates, silicates, etc. together with the hydroxyl ions in free state. However, most of the waters are rich in carbonates with little concentration of other alkalinity imparting ions.

Total alkalinity, carbonates and bicarbonates can be estimated by titrating the sample with a strong acid (HCl or H_2SO_4), first to pH 8.3 using phenolphthalein as an indicator and then further to pH between 4.2 and 5.4 with methyl orange or mixed indicator. In first case, it is total alkalinity (TA). Values of carbonates, bicarbonates and hydroxyl ions can be completed from these two types of alkalinities.

Reagents

A. Hydrochloric acid, 0.1 N

Dilute 12N concentrated HCl (sp. gr. 1.18) to 12 times (8.34 → 100ml) to prepare 1.0N HCl. Dilute it further to make 0.1N HCl (100 →1000ml). Standardize it against sodium carbonate solution.

B. Methyl orange indicator, 0.05%

Dissolve 0.5g of methyl orange in 100 ml of distilled water.

C. Phenolphthalein indicator

Dissolve 0.5g of phenolphthalein in 50 ml of 95% ethanol and 50ml of distilled water. Add 0.05N CO_2 free NaOH solution dropwise, until the solution turns faintly pink.

D. Sodium carbonate, 0.1N

Dissolve 5.300 g of $NaCo_3$, previously dried at 250^{o}Cfor about 4 hours, in distilled water to prepare 1 litre of solution.

Procedure

1. Take 100ml of sample on conical flask and add 2 drops of phenolphthalein indicator.
2. If the solution remains colourless, PA = O, and total alkalinity is determined as described in step 4.
3. If the colour changes to pink after addition of phenolphthalein, titrate it with 0.1 N HCl until the colour disappears at end point. The phenolphthalein alkalinity (PA).
4. Now add 2 – 3 drops of methyl orange to the same sample and continue the titration further, until the yellow colour changes to pink at end point. This is total alkalinity (TA).

Calculation

$$\text{PA as } CaCO_3, \text{ mg/L} = \frac{(A \times \text{Normality}) \text{ of HCl} \times 1000 \times 50}{\text{ml of sample}}$$

$$\text{TA as } CaCO_3, \text{ mg/L} = \frac{(B \times \text{Normality}) \text{ of HCl} \times 1000 \times 50}{\text{ml of sample}}$$

where, A = ml of HCl used with only phenolphthalein

B = ml of total HCl used with phenolphthalein and methyl orange.

PA = phenolphthalein alkalinity

TA = total alkalinity

Concentration of carbonates, bicarbonates and hydroxyl ions can be determined from the table using data of PA and TA.

Table : Values of hydroxyl ions, carbonates and bicarbonates from the values pf phenolphthalein and total alkalinities.

Result of titration	**OH alkalinity as $CaCO_3$**	**CO_3 alkalinity as $CaCO_3$**	**HCO_3 alkalinity as $CaCO_3$**
P = 0	0	0	T
P < ½ T	0	2P	T – 2P
P = ½ T	0	2P	0
P = ½ T	2P – T	2(T – P)	0
P > ½ T	2P – T	2(T – P)	0
P = T	T	0	0

Where, P = Phenolphthalein alkalinity

T = Total alkalinity

Acidity

Titration Method

Principle

Acidity of the water is its capacity to neutralize a strong base to a fixed pH. It is caused by the presence of strong mineral acids, weak acids and hydrolyzing salts of strong acids. However, in natural unpolluted freshwater, the acidity is mostly due to presence of free CO_2 in the form of carbonic acid.

Acidity can be determined by filtering the sample with a strong base such as NaOH using methyl orange or phenolphthalein as an indicator. If the sample has strong mineral acids and their salts, it is titrated first to pH 3.7, using methyl orange as indicator. This is called as *methyl orange acidity*. If the sample is titrated directly to pH 8.3 using phenolphalein, the resultant value is the *total acidity*. The results of acidity should always be accompanied by the end point pH.

The titration method is suitable mainly for the samples which are colourless. The original colour of the sample may interface with indicator colours.

Procedure

1. Take 100 ml of colourless sample in a conical flask and add 2 – 3 drops of methyl orange indicator.
2. If the solution turns yellow, the methyl orange acidity is absent. In case the contents turn pink, titrate with 0.05 N NaOH. At the end point colour changes from pink to yellow.
3. Now add a few drops of the phenolphthalein indicator to the same sample and titrate further with NaOH until the contents turn pink.

Calculation

$$\text{Methyl orange acidity, mg/L} = \frac{\text{A x N of NaOH x 1000 x 50}}{\text{ml of sample}}$$

$$\text{Phenolphthalein acidity, mg/L} = \frac{\text{B x N of NaOH x 1000 x 50}}{\text{ml of sample}}$$

$$\text{Total acidity, mg/L} = \frac{\text{(A + B) x N of NaOH x 1000 x 50}}{\text{ml of sample}}$$

where, A = volume of NaOH used with methyl orange in titrating the sample to pH 3.7

B = Volume of NaOH used with phenolphthalein in titrating the sample from pH 3.7 to pH 8.3.

Carbon Dioxide

Principle

Free CO_2 can be determined by titrating the sample using a strong alkali (such as carbonate free NaOH) to pH 8.3. At this pH all the free CO_2 is converted into bicarbonates.

Reagents

A. Sodium hydroxide, 0.05N

Prepare 1.0 N NaOH by dissolving 40g of NaOH in Co_2 free distilled water (boiled) to make 1 litre of solution. Dilute 50 ml of 1.0 N NaOH to 1 litre. Standardize H_2SO_4, HCl or oxalic acid.

B. Phenolphthalein indicator

See alkalinity determination

Procedure

1. Take 100 ml of sample in a conical flask and add few drops of phenolphthalein indicator.
2. If the colour turns pink, free CO_2 is absent. If the sample remains colourless titrate it against 0.05 N NaOH. At the end point a pink colour appears.

Calculation

$$\text{Free } CO_2, \text{ mg/L} = \frac{\text{(ml x N) of NaOH x 1000 x 44}}{\text{ml of sample}}$$

Calcium

Principle

Many indicators such as ammonium purpurate, calcon etc. form a complex with only calcium but not with magnesium at higher pH. As EDTA is having a higher affinity towards calcium; the former complex is broken down

and a is formed. However, EDTA has a properly to combine with both Ca^{++} and Mg^{++}. Therefore magnesium is largely precipitated as its hydroxide at higher pH.

Reagents

A. EDTA solution, 0.01 M

See hardness determination

B. Sodium hydroxide, 1N

Dissolve 40 g of NaOH in distilled water and dilute to 1 litre.

C. Murexide indicator

Mix 0.2g of ammonium purpurate with 100g of NaCl (A.R.) and grind.

Procedure

1. Take 50 ml of sample in a conical flask. If the sample is having higher alkalinity, use smaller volumes diluted to 50ml.
2. Add 2.0ml of NaOH solution in the sample.
3. Add 100 to 200mg of murexide indicator a pink colour develops.
4. Titrate against EDTA solution until the pink colour changes to purple. For better judgement of end point, compare the purple colour with the distilled water blank titration end point.

Calculation

$$\text{Calcium, mg/L} = \frac{X \times 400.8}{\text{ml of sample}}$$

where, X = Volume of EDTA used.

Magnesium

Principle

Calcium and magnesium form a complex of wine red colour with Eriochrome Black T at pH 10.0. The EDTA has got a stronger affinity for Ca^{++} and Mg^{++}; the former complex is broken down and a new complex of blue colour is formed. The value of Mg^{++} can be obtained by subtracting the value of calcium from the total of Ca^{++} + Mg^{++}.

Reagents

A. EDTA solution, 0.01 M

See hardness determination

B. Buffer solution

See hardness determination

C. Eriochrome Black T indicator

See hardness determination.

Procedure

1. Find out the volume of EDTA used in calcium determination.
2. Also find out the volume of EDTA used in hardness (Ca^{++} + Mg^{++}) determination with same volume of the sample as taken in the calcium determination.

Calculation

$$\text{a)} \quad Mg^{++}, \text{ mg/L} = \frac{y - x \times 400.8}{\text{Volume of sample} \times 1.645}$$

Where, y = EDTA used in hardness determination

x = EDTA used in calcium determination for the same volume of the sample.

b) Mg^{++}, mg/L = Total hardness (as mg/L $CaCO_3$) – calcium hardness (as mg/L $CaCO_3$) X 0.244 where, Calcium hardness (as mg/L $CaCO_3$) = Ca, mg/L X 2.497

Iron

Principle

All the iron is converted into ferrous state by boiling hydrochloric acid and hydroxylamine. The reduced iron chelates with 1, 10 – phenothroline at pH 3.2 to 3.3 to form a complex of orange – red colour. The intensity of this colour is proportional to the concentration of iron and follows Beer's law, and therefore, can be determined colorimetrically.

Strong oxidizing agents like cyanide, nitrate and phosphates (mainly polyphosphates) chromium, zinc, cobalt, copper and nickel interfere with the determination of iron. The boiling of the sample with an acid initially removes interference of cyanide, nitrate and polyphosphates. Hydroxylamine eliminates the interference caused by strong oxidizing agents. Bismuth, cadmium, mercury and silver precipitate the phenonthroline, and the errors caused by them and other metals can be overcome by using excess of phenonthroline.

Reagents

A. Hydrochloric acid, conc.

B. Hydroxylamine hydrochloride solution

Dissolve 10g of hydroxylamine hydrochloride NH_2OH. HCl in distilled water to prepare 100 ml of solution (stable for several months)

C. Ammonium acetate buffer solution

Dissolve 250g of NH_4CH_3COOH in 150ml distilled water and add 700ml glacial acid.

D. Phenonthroline solution

Dissolve 100mg of 1, 10 – phenonthroline monohydrate in 100ml distilled water by through stirring and heating upto 80°C (should not be boiled). Never use magnetic stirrer, with a piece of iron in the solution.

E. Stock Iron solution (200mg Fe/L)

Add 20ml conc. H_2SO_4 to 50 ml distilled water and dissolve 1.404g of ferrous ammonium sulphate [$Fe(NH_4)_2(SO_4)_2.6H_2O$]. Add 0.1 N$KMnO_4$ slowly till a persistent faint colour appears. Dilute to 1 litre.

F. Standard iron solution (10mg Fe/L)

Dilute the stock Fe solution 20 times with distilled water (50 → 1000ml).

Procedure

1. Take 50ml of sample or an aliquot, containing not more than 4mg/L of iron in a 150ml conical flask.
2. Add 2 ml conc. HCl and 1ml of hydroxylamine hydrochloride solution.
3. Boil the contents to half of the volume for dissolution of all the iron.
4. Cool and add 10ml ammonium acetate buffer and 2ml phenothroline solution. If the sample contains interference of heavy metals, add 10ml of phenonthroline instead of 2ml. an orange – red colour appears.
5. Make up the volume to 100ml, and after 10 minutes take the reading at 510nm on a spectrophotometer.
6. Prepare a standard curve in the range of 1 to 4 mg/L of iron using various dilutions of standard iron solution.
7. If only the solution iron is to be determined, acidify the sample directly with 1ml conc. HCl and proceed from the step 4 onwards.
8. Calculate the concentration of Fe directly from the standard curve.

Manganese

Mn compounds present in chemical waste waters and in drainage from coal industries. They react with alkalinity in receiving waters in the presence of air forming characteristic brown precipitate, manganic hydroxide. The heptavalent permanganate ion is used to oxidize Mn.

(i) Mn may be measured by AAFS. At pH 9 – 11, solvent extraction is made with 1% oxine. Air – acetylene flame is used (λ = 279.5 nm).

(ii) Persulfate method : Soluble Mn compounds are oxidized to permanganate in acid solutions in presence of $AgNO_3$ Resulting colour of permanganate solution, stable for at least 24 h if excess persulfate is used, measured photometrically at 525 nm.

$$2MnSO_4 + 5(NH_4)_2S_2O_8 + 10HNO_3 + 8H_2O \rightarrow 2HMnO_4 + 12H_2SO_4 + 10NH4NO_3$$

Chloride and ferric ions interfere. Chloride is eliminated by adding $HgSO_4$. Br^- and I^- still interfere but only trace amounts will be present/ Phosphoric acid eliminates Fe^{3+}.

Mercury

Very toxic. A major source is the production of chlorine where Hg is used as an electrode in the electrolysis of brine. Pulp and paper, oil refining and plastic industry and battery manufacturing industries also contribute to Hg pollution. Because Hg can be lost readily from samples, they are preserved by treating with HNO_3 to reduce pH to 2.

(i) Flameless Atomic Absorption Method : Specific technique for Hg. At room temperature Hg is reduced Hg^o (elemental) by $SnCl_2$. Sweeped Hg by air into an absorption cell. Hg can be estimated by absorbance at 253.7 nm.

(ii) Dithizone method : 2 – 10 mg Hg can be estimated by this method. Hg^{2+} reacts with dithizone in 1 NH_2SO_4 at a pH of about 1 to form an orange colour, measured photometrically at 490 nm. Mercury dithizonate must be measured quickly because it is photosensitive Cu, Au, Pd, Pt(II) and Ag mostly interfere.

Zinc

Essential beneficial element in human growth. At higher concentrations cause bitter state. The major industrial wastes contributing to Zn pollution are galvanizing wastes, Zn platting wastes, viscose rayon wastes, dye and rubber factory wastes.

(i) Dithizone Method : Nearly 20 metals can react with dithizone to produce coloured coordination compounds. Most of the interferences can be eliminated by complexing with thiosulfate and adjusting the pH to 4.0 to 5.5. Zn dithizonate is measured at 535 nm against a reagent blank. Visual comparison can also be made.

(ii) Zincon Method : At pH 9, zinc reacts with 2 – carboxy – 2' – hydroxyl – 5' – sulfoformazyl benzene (Zincon) to form a blue complex. Since other heavy metals can also form coloured complexes with zincon they must be eliminated before analysis. Measured the absorbance at 620 nm.

Ammonia

Ammonia of mineral is rare in natural waters. The most important source of ammonia is the ammonification of organic matter. Sewage has large quantities of nitrogenous matter, thus its disposal tends to increase the ammonia content of the waters. Occurrence of ammonia in the waters can be accepted as the chemical evidence of organic pollution. If only ammonia is

present, pollution by sewage must be very recent. The occurrence of NO_2 with ammonia indicates that some time has been lapsed since the pollution has occurred. If all the nitrogen is present in nitrate form, a long time has been passed after pollution because water has purified itself and all nitrogenous matter has been oxidized.

I. Colorimetric method

Principle

Ammonium ion reacts with Nessler's reagent (K_2HgI_4) to form a brown colour substance and can be determined colorimetrically. Most of natural waters and waste waters have interfering substances; therefore, the steam distillation of ammonia becomes essential.

Apparatus and Reagents

A. Distillation unit

There are a number of designs available. Figures shows the most common distillation assembly, also known as micro Kjeldahl distillation unit.

B. Standard ammonia solution

Dissolve 3.819 g of anhydrous NH_4Cl in distilled water to prepare 1 litre of solution. This solution contains 1000 mg/LNH_3_N. Dilute this solution 100 times (10 $\rightarrow$ 1000 ml) to prepare the solution containing 10 mg/L NH_3_N.

C. Sulphuric acid, 0.04 N

Add 2 ml of (1 + 1) H2SO4 (sp. Gr. 1.84) to 1 litre of distilled water.

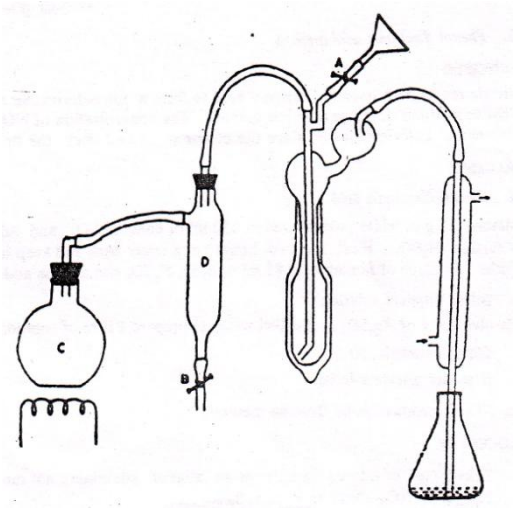
D. Borax buffer

Add 4 g of $Na_2B_4O_7$. $10H_2O$ to 100 ml of distilled water. Heat to dissolve the crystals.

E. Nessler's reagent

a) Dissolve 25 g of HgI_2 and 20 g of KI in 500 ml of distilled water.

b) Dissolve 100 g of NaOH in 500 ml of distilled water. Store these two solutions in brown glass air tight stoppered bottles. Mix (1+1) just before us.



Procedure

1. Take 50 ml of sample in the distillation assembly through tap A and add 1 ml of borax buffer solution.
2. Put 2.5 ml of 0.4 N H_2SO_4 in a 100 ml conical flask and place below the condenser so that the tip pf outlet dips in the acid.
3. Keep the boiling flask (C) on the heater to pass the steam into the sample through chamber (D).
4. Ammonia will be distilled off and collects in the sulphuric acid as $(NH_4)_2 SO_4$. Continue the distillation until nearly 40ml of distillate is collected.
5. Remove the flask having distillate. Cool the boiling flask so that all the waste contents will be sucked into the chamber (D).
6. Remove the waste contents through tap B.
7. Run a blank with distilled water using same quantity of the chemicals.
8. Make up the volume of distillate to 50 ml and add 1 ml of Nessler's reagent. A brown colour will develop.

9. Measure the absorbance at 425 nm.
10. Prepare a standard curve between 0.05 to 2.0 mg/L of NH_3 _N by diluting the standard NH_2_N solution.

Calculation

Find out the concentration of NH_3_N directly from the standard curve.

Nitrite

There are no mineral sources of this ion in natural waters. Nitrite represent an intermediate form during denitrification and nitrification reactions in nitrogen cycle. Nitrite is a very unstable ion and gets converted into either ammonia or nitrate depending upon the conditions prevailing in the water. Presence of even a small quantity of nitrite will indicate the organic pollution and the availability of partially oxidized nitrogenous matter. Nitrites may also be produced in distribution systems through the activities of microorganisms on ammonia. Another source of nitrite entering the system may be its use as corrosion inhibitor in industrial process waters. The high concentration of nitrites can also cause 'Blue – baby' disease (Methamoglobinemia) in infants.

Principle

Nitrite forms a diazonium salt with sulphanilic acid in acid medium (2.0 – 2.5 pH), with combines with α - naphthylamine hydrochloride to form a pinkish dye. The colour so produced obeys Beer's law and can be determined colorimetrically.

Reagents

A. Disodium ethylene diamine tetra acetic acid (Na_2EDTA) solution

Dissolve 500 mg of disodium salt EDTA in distilled water to make 100 ml solution.

B. Sulphanilic acid Solution

Dissolve 600 mg of sulphanilic acid in 70 ml of hot distilled water and add 20 ml of concentrated HCl after cooling and dilute to 100 ml.

C. α - naphthylamine hydrochloride solution

Dissolve 100 mg of α - naphthylamine hydrochloride in distilled water to which 1 ml concentrated HCl has been added. Dilute the contents to 100 ml and place in a cool place. If a precipitate occurs after few days, the reagent can be used further by filtering the solution.

D. Sodium acetate solution

Dissolve 16 g of anhydrous CH_3COONa or 27.2 g of CH_3COONa . $3H_2O$ in distilled water to prepare 100 ml solution.

E. Standard nitrite solution (1mg/L NH_2_N)

Dissolve 1.232 g $NaNO_2$ in distilled water and dilute to litre (250 mg/L NO_2_N). Dilute this solution 250 times (4 → 1000 ml) to prepare standard solution having 1 mg/L NO_2_N.

Procedure

1. Take 50 ml of colourless filtered sample not having more than 1.0 mg/L NO_2_N in a conical flask. The colour can be removed by activated charcoal in case of coloured samples.
2. Add 1 ml of each EDTA, sulphanilic acid, α - naphthylamine hydrochloride and sodium acetate solutions in sequence.
3. A wine red colour will appear in the presence of nitrites. Take the reading at 520 nm.

4. Compare the absorbance with the standard curve to calculate the nitrite content.
5. Prepare the standard curve between 0.0 to 1.0 mg NO_2 . N/L at the interval of 0.1 employing the same procedure as for the sample.

Nitrate

Nitrate represents the highest oxidized form of nitrogen. Only a few mineral sources of nitrate such as soda niter deposits in Chile, exist in nature and most of the surface water are therefore, deficient in nitrate. The most important source of the nitrate is biological oxidation of organic nitrogenous substances which come in sewage and industrial wastes or produced indigenously in the waters. Domestic sewage contains very high amounts of nitrogenous compounds. Run – off from agricultural fields is also high in nitrate. Atmospheric nitrogen fixed into nitrates by the nitrogen fixing organisms is also a significant contributor to nitrates in the waters.

Many ground waters have significant quantities of nitrates due to leaching of the nitrate with the percolating water. Ground water can also be contaminated by sewage and other wastes rich in nitrates. High amounts of nitrates are generally indicative of pollution.

Nitrates are of prime concern because of methamoglobinemia when the concentration of nitrates exceeds 40 mg/L. In this disease the skin becomes blue due to decreased efficiency of haemoglobin to combine with oxygen. In cattle, the high concentration of nitrates is reported to cause more mortality in pigs and calves and abortion in brood animals.

I. BRUCINE METHOD

Principle

Nitrate and brucine react to produce a yellow colour, the intensity of which can be measured at 410 nm. The reaction is highly dependent upon the

heat generated during the test. However, it can be controlled by carrying out the reaction for a fixed time at a constant fixed temperature. The method is suitable for the sample having a very wide range of salinity.

REAGENTS

A. Brucine – sulfanilic acid solution

Dissolve 1 dg brucine sulphate and 0.1 g of sulfanilic acid in about 70 ml of hot distilled water. After addition of 3 ml conc. HCl, make up the volume to 100 ml. The pink colour develops slowly, does not effect the sensitivity.

B. Sulphuric acid solution

Add 500 ml conc. H_2SO_4 in 125 ml distilled water and cool.

C. Sodium chloride solution

Dissolve 300 g NaCl in distilled water and dilute to 1 litre.

D. Sodium arsenite solution

Dissolve 0.722 g of KNO_3 in distilled water and make up the volume to 1 litre. This solution contains 100 mg N/L. Dilute it to 100 times to prepare a solution having 1 mg N/L (10 $\rightarrow$ 1000 ml).

Procedure

1. Free chlorine interferes with the nitrate determination. If the sample is having residual chlorine, remove it by addition of 0.05 ml (one drop) of sodium arsenite solution for each 0.1 mg of chlorine. Add one drop in excess to a 50 ml sample portion.
2. Take 10 ml of sample or an aliquot diluted to 10 ml in a 50 ml test tube.
3. Put all the tubes in a wire rack.
4. Place the rack in cool water bath and add 2 ml of NaCl solution.

5. Add 10 ml of H_2SO_4 solution after mixing the contents thoroughly swirling by hand.
6. Add 0.5 ml brucine reagent and mix thoroughly.
7. Place the rack in a hot water bath with boiling water, exactly for 20 minutes.
8. Cool the contents again in a cold water bath and take the reading at ******* nm.
9. Find out the concentration of NO_3_N from the standard curve.
10. Prepare a standard curve between concentration and absorbance by taking the dilutions from 0.1 to 1.0 mg N/L at the interval of 0.1, employing the same procedure as for the sample.

Cyanides

'Cyanide' refers to all of the CN groups in cyanide compounds that can be determined as the cyanide ion, CN^- by the methods used. Free cyanide (CN^- or HCN) is very toxic pollutant arising from metal refining, cleaning, electro plating and various industrial processes. Thiocyanate (SCN^-) is not very toxic to aquatic life. However, upon chlorination toxic CNCl is formed. Mainly sulphides giving rise to H_2S during distillation interfere with Cyanide analysis. Removed by treating with Cd $(NO_3)_2$ solution and the precipitate is filtered off.

250 – 500 ml of sample is treated with 50 ml NH_2SO_4 and 20 ml of 51% $MgCl_2$. $6H_2O$. SCN^- is removed. Refluxed and the HCN is collected in 500 ml of 1 N NaOH in a gas washer.

Measurement of CN^-

(i) Titrimetric method : This method is suitable for cyanide concentrations above 1 mg/l CN^- in the alkaline distillate is titrated with std $AgNO_3$ solution. The soluble cyanide complex $Ag(CN)_2^-$ formed is treated with silver sensitive indicator p – dimethylamino – benzalrhodanine solution. End point is the

colour change from yellow to a salmon colour. It is compared with a blank under the same conditions.

(ii) Spectrophotometric method : Chloramine – T converts CN^- in the alkaline distillate to CNCl at pH < 8 without hydrolyzig to CN^-, Pyridine barbituric acid reagent is added and the red – blue dye formed is measured at 578 nm.

(iii) Ion selective Electrode : CN^- in alkaline distillate can be determined potentiometrically using a CN^- selective electrode in combination with a double junction reference electrode and a pH meter. This method is suitable if the concentration range is 0.05 to 10 mg CN^- / l. **Sulphate**

It is a naturally occurring anion in all kinds of natural waters. In arid and semiarid regions, it is found in particularly higher concentrations due to the accumulation of soluble salts in soils and shallow aquifers. Biological oxidation of reduced sulphur species to sulphate also increases its concentration. Rain water has quite high concentration of sulphate particularly in the areas with high atmospheric pollution. Discharge of industrial wastes and domestic sewage in waters tends to increase its concentration. Most of the salts of sulphate are soluble in water and as such it is not precipitated. However, it may undergo transformations to sulphur and hydrogen sulphide depending upon the redox potential of the water.

Sulphate is an important constituent of hardness with calcium and magnesium. Sulphate produces an objectionable taste at 300 – 400 mg/L concentrations. Above 500 mg/L, a bitter taste is produced in the water. At concentrations around 1000 mg/L, it is laxative (U.S. EPA, 1973). Sulphate with sodium interferes with the normal functioning of the intestine. It has got some indirect importance by producing H_2S which helps in corrosion of concrete, thus posing serious problems in the waste water collection pipes.

SULPHATE

Turbidimetric method

Principle

Sulphate ion is precipitated in the form of barium sulphate by adding barium chloride in hydrochloric acid medium. The concentration of the sulphate can be determined from the absorbance of the light by barium sulphate and then comparing it with a standard curve.

Suspended matter and original colour of the sample may interfere with the sulphate determination. Suspended matter can be removed by filtration. Presence of silica in excess of 500 mg/L and large quantity of organic matter may affect the satisfactory precipitation of barium sulphate.

REAGENTS

A. Conditioning reagent

Mix 75 g of NaCl, 30 ml conc. HCl, 100 ml 95% ethyl or isopropyl alcohol in 300 ml distilled water. Add 50 ml glycerol to this solution and mix thoroughly.

B. Barium chloride

Crystals of $BaCl_2$.

C. Standard sulphate solution

Dissolve 0.1479 g of anhydrous Na_2SO_4 in distilled water to make 1 litre of solution. This solution contains 100 mg/L of sulphate. b) Standard solution can also be prepared by diluting 10.41 ml of 0.02 NH_2SO_4 to 100 ml with distilled water. It also contains 100 mg/L of sulphate.

Procedure

1. 100 ml of sample (not containing more than 40 mg/L of SO_4) or a suitable aliquot diluted to 100 ml in 250 ml conical flask and add 0.5 ml of conditioning reagent.
2. Stir the sample on a magnetic stirrer and during stirring, add a spoonful of $BaCl_2$, crystals : Stir for only 1 minute after addition of $BaCl_2$ crystals.
3. Take the reading on a spectrophotometer at 420 nm exactly after 4 minutes; Find out the concentration of sulphate from the standasr curve.
4. Prepare the standard curve; employing the same procedure as described above, for 0.0 – 40.0 mg/L at the interval of 5 mg/L.

Sulphide

Trace amounts of sulphides are present in ground and surface water due to reduction of sulphate. It is found in higher concentrations when organic matter is high and redox potential is low (reducing conditions). Microorganisms use sulphate as electron acceptor to decompose organic matter and convert sulphate into sulphide. Certain microorganisms are also capable of reducing elemental sulphur into sulphide. IT is therefore considered as indicative of organic pollution and prevailing reducing conditions.

It has got an objectionable odour resembling to that of rotten eggs which decreases the aesthetic value and renders the water unfit for consumption. If the sufficient iron is present in reducing conditions, sulphide may be precipitated as ferrous sulphide decreasing the concentration of both iron and sulphur in water. As such it is a highly soluble gas but the solubility decreases with the increase in concentration of oxygen.

Hydrogen sulphide has some toxic effects on the organisms present in the water. It is highly corrosive to the concrete and metals and poses problems in sewage collection systems. H_2S also promotes growth of certain bacteria responsible for clogging of pipes and screens.

Chlorides

Chlorides occur naturally in all types of waters. In natural freshwaters, however, its concentration remains quite low and is generally less than that of sulphates and bicarbonates. The most important source of chlorides in the waters is the discharge of domestic sewage. Man and other animals excrete very high quantities of chlorides together with nitrogenous compounds. About 8 – 15 grams of NaCl is excreted by a person per day. Therefore, the chloride concentration serves as an indicator of pollution by sewage. Industries are also important sources of chlorides.

Chlorides are highly soluble with most of the naturally occurring cations and does not precipitate, sedimented and cannot be removed biologically in treatment of the wastes,

It is harmless upto1500 mg/L concentration but produces a salty taste at 250 – 500 mg/L level. It can also corrode concrete by extracting calcium in the form of calcide. Magnesium chloride water generates hydrochloric acid after heating, which is also highly corrosive and create problems in boilers.

Principle

Silver nitrate reacts with chloride to form very slightly soluble white precipitate of AgCl. At the end point when all the chlorides get precipitated, free silver ions react with chromate to form silver chromate of reddish brown colour.

REAGENTS

A. Silver nitrate, 0.02N

Dissolve 3.400 g of dried $AgNO_3$ (A. R.) in distilled water to make 1 litre of solution and keep in a dark bottle.

B. Potassium chromate, 5%

Dissolve 5 g of K_2CrO_4 in 100 ml of distilled water.

Procedure

1. Take 50 ml of sample in a conical flask and add 2 ml of K_2CrO_4 solution.
2. Titrate the contents against 0.02 N $AgNO_3$ until a persistant red tinge appears.

Calculation

$$\text{Chloride, mg/L} = \frac{(\text{ml} \times \text{N}) \text{ of } AgNO_3 \times 1000 \times 35.5}{\text{ml sample}}$$

Fluoride method

F^- level of 1 mg/l effectively prevents dental caries without harmful effects on health. When the fluoride level exceeds the limits, fluorosis occurs.

(i) Electrode method : This method is suitable for fluoride concentrations from 0.1 to more than 10 mg/l. Fluoride electrode measures the ion activity of fluoride ion in solution rather than concentration. Electrode consists of a laser – type doped lanthanum fluoride crystal. The potentials established by fluoride solutions of different concentrations are measured. From the potentials measured for each sample, corresponding fluoride concentrations are determined from the potential vs concentration standard curves.

(ii) Complexone method : Distilled sample is treated with alizarin fluorine blue – lanthanum reagent to form a blue complex that is measured colorimeterically at 620 nm. Distillation removes most of the interferences related with fluoride determination.

Dissolved Oxygen

Dissolved oxygen is one of the most important parameters in water quality assessment and reflects the physical and biological processes prevailing in the waters. Its presence is essential to maintain the higher forms of

biological life in the water and the effects of a waste discharge in a water body are largely determined by the oxygen balance of the system. Non – polluted surface waters are normally saturated with dissolved oxygen. Oxygen can be rapidly removed from the waters by discharge of the oxygen demanding wastes. Other inorganic reductants such as hydrogen sulphide, ammonia, nitrites, ferrous iron, and other oxidizable substances also tend to decrease dissolved oxygen in water.

Low oxygen in water can kill fish and other organisms present in water. Organisms have specific requirement of oxygen, for example, game fish requires at least 5 mg/L and coarse fish about 2 mg/L of minimum dissolved oxygen in water.

The concentration of oxygen will also reflect whether the processes undergoing are aerobic or anaerobic. Low oxygen concentrations are generally associated with heavy contamination by organic matter. In such conditions oxygen, sometimes, totally disappears from the water.

I. Winkler's Iodometric method

Principle

The manganous sulphate reats with the alkali (KOH or NaOH) to form a white precipitate of manganous hydroxide which in the presence of oxygen, gets oxidized to a brown colour compound. In the strong acid medium manganic ions are reduced by iodide ions which get converted into iodine equivalent to the original concentration of oxygen in the sample. The iodine can be titrated against thiosulphate using starch as an indicator.

REAGENTS

A. Sodium thiosulphate, 0.025 N

Dissolve 24.82 g of $Na_2S_2O_3$ $5H_2O$ in boiled distilled water and make up the volume to 1 litre. Add 0.4 g of borax or a pallet of NaHO as stabilizer. This is 0.1 N stock solution. Dilute it to 4 times with boiled distilled water to prepare 0.025 N solution (250 → 1000 ml). Keep in a brown glass stoppered bottle.

B. Alkaline potassium iodide solution

Dissolve 100 g of KOH and 50 g of KI in 200 ml of boiled distilled water.

C. Manganous sulphate solution

Dissolve 100 g of $MnSO_4$ $4H_2O$ in 200 ml of boiled distilled water and filter.

D. Starch solution

Dissolve 1 g of starch in 100 ml of warm (80^oC – 90^oC) distilled water and add a few drops of formaldehyde solution.

E. Sulphuric acid

H_2SO_4 conc. (sp. gr. 1.84)

Procedure

1. Fill the sample in a glass stoppered bottle (BOD Bottle) of known volume (100 – 300 ml) carefully, avoiding any kind of bubbling and trapping of the air bubbles in the bottle after placing the stopper.
2. Pour 1 ml of each $MnSO_4$ and alkaline KI solutions (in case; the volume of the sample is about 300 ml, instead of 1 ml of reagents and 2 ml solutions of each), well below the surface front the walls. The reagents can also be poured at the bottom of the bottle will the help of special pipette syringes to

ensure better mixing of the reagents with the sample. Use always, separate pipettes for these two reagents. A precipitate will appear.

3. Place the stopper and shake the contents well by inverting the bottle repeatedly. Keep the bottle for some time to settle down the precipitate. If the titration is to be prolonged for few days, keep the sample at this stage with the precipitate.
4. Add 1 – 2 ml of concentrated H_2SO_4 and shake well to dissolve the precipitate.
5. Remove either the whole contents, or a part of them (5 – 100 ml) in a conical flask for titration. Prevent any bubbling to avoid further mixing of oxygen.
6. Titrate the contents, within one hour of dissolution of the precipitate against sodium thiosulphate solution using starch as an indicator. At the end point, initial dark blue colour changes to colourless.

Calculation

When whole contents have been titrated :

$$\text{Diss. Oxygen, mg/L} = \frac{\text{(ml x N) of titrant x 8 x 1000}}{V_1 - V}$$

When only a part of the contents has been titrated :

$$\text{Diss. Oxygen, mg/L} = \frac{\text{(ml x N) of titrant x 8 x 1000}}{V_2 \frac{V_1 - V}{V_1}}$$

Where, V_1 = volume of sample bottle after placing the stopper.

V_2 = volume of the part of the contents titrated.

V = volume of MnSO4 and KI added.

In oceanography, the unit ml/L is preferred over mg/L. It can be obtained by dividing the value in mg/L by 1.43.

Biochemical Oxygen Demand (B.O.D)

BOD is the amount of oxygen utilized by microorganisms in stabilizing the organic matter. On an average basis, the demand for oxygen is proportional to the amount of organic waste to be degraded aerobically. Hence, BOD approximates the amount of oxidizable organic matter present in the solution and the BOD value can be used as a measure of waste strength. It is highly important to know the amount of organic matter present in the waste treatment system and that the quantity of oxygen required for its stabilization. The BOD values are thus very useful in process design and loading calculations as well as the measure of treatment plant efficiency and operation. The BOD test is also useful in stream pollution control management and in evaluating the self purification capacities of streams which serves as a measure to assess the quantity of wastes which can be safely assimilated by the stream.

The complete degradation of the organic matter may take as long as 20 to 30 days. Simple organic compounds like glucose are almost completely oxidized in 5 days; while domestic sewage by only about 65% and complex organic compounds might be oxidized only upto 40% in this period. The 20 – 30 days period is of less significance in practice, therefore, the BOD test has been developed for 5 days at $20^{o}C$.

Types of microorganisms, pH, presence of toxins, some reduced mineral matter, and nitrification process are the important factors influencing the BOD test.

BOD in general gives a qualitative index of the organic substances which are degraded quickly in a short period of time. BOD values should not be used

as equivalent to the organic load regardless of the presence of non degradable organic matter, presence of toxins and local changes in populations of microorganisms. BOD test should be restricted to only suitable wastes in management of the treatment paints, however, for other kinds of wastes chemical oxygen demand (COD) values may be more appropriate.

PRINCIPLE

Biochemical Oxygen Demand (BOD) is the measure of the degradable organic material present in a water sample, and can be defined as the amount of oxygen required by the microorganisms in stabilizing the biologically degradable organic matter under aerobic conditions.

The principle of the method involves, measuring the difference of the oxygen concentration between the sample and after incubating it for 5 days at 20^oC.

APPARATUS AND REAGENTS

A. BOD bottles

B. BOD incubator

Having temperature control at 20^oC.

C. Phosphate buffer

Dissolve each 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g Na_2HPO_4 . $7H_2O$ and 1.7 g NH4Cl in distilled water to prepare 1 liter of solution. Adjust pH to 7.2.

D. Magnesium sulphate

Dissolve 82.5 g $MgSO_4.7H_2O$ in distilled water to prepare 1 litre of solution.

E. Calcium chloride

Dissolve 27.5 g of anhydrous $CaCl_2$ in distilled water to prepare 1 litre of solution.

F. Ferric chloride

Dissolve 0.25 g of $FeCl_3.6H_2O$ in distilled water to prepare 1 litre of solution.

G. Sodium Sulphite solution, 0.025 N

Dissolve 1.575 g Na_2SO_4 and dilute to 1000 ml. Solution should be prepared freshly.

PROCEDURE

1. Prepare dilution water in a glass container by bubbling compressed air in distilled water for about 30 minutes.
2. Add 1 ml each of phosphate buffer, magnesium sulphate, calcium chloride, and ferric – chloride solutions for each litre of dilution water and mix thoroughly.
3. Neutralize the sample to pH around 7.0 by using 1 N NaOH or H_2SO_4.
4. Since the DO in the sample is likely to be exhausted; it is usually necessary to prepare a suitable dilution of the sample according to the expected BOD range. See table for dilution of the sample.
5. Prepare dilutions I a bucket or a large glass trough, mix the contents thoroughly. Fill 2 sets of the BOD bottles.
6. Keep one set of the bottles in BOD incubator at 20^oC for 5 days and determine the DO content in another set immediately.
7. Determine DO in the sample bottles, immediately after the completion of 5 days incubation.
8. Similarly for blank, take 2 BOD bottles for dilution water. In one, determine the DO content and the other incubate with the sample to determine. DO after 5 days.

Table : Preparation of dilutions for various ranges of BOD in the sample

Range of BOD mg/IO_2	Dilution (%)	Sample of volume in 1 litre of mixture
0 – 6	No dilution	1000
4 – 12	50	500
10 – 30	20	200
20 – 60	10	100
40 – 120	5	50
100 – 300	2	20
200 – 600	1	10
400 – 1200	0.5	5
1000 – 3000	0.2	2
2000 – 6000	0.1	1
Above 6000	0.05	0.5

Calculation

BOD, mg/L = (D_0 – D_5) x dilution factor

Where, D_0 = Initial DO in the sample

D_5 = DO after 5 days

Chemical Oxygen Demand (COD)

Chemical oxygen demand is the oxygen required by the organic substances in water to oxidize them by a strong chemical oxidant. The COD usually refers to the laboratory dichromate oxidation procedure.

The determinations of COD values are of great importance where BOD values cannot be determined accurately due to the presence of toxins and other such unfavourable conditions for growth of microorganisms. The main disadvantage of the test is that oxygen is also consumed by the oxidation of inorganic matter such as nitrites, sulphides, reduced metal ions, thiosulphate, etc., and some organic materials are not oxidized by dichromate method such as benzene, pyridine and few other cyclic organic compounds. Consequently, the test is a poor measure of strength of organic wastes unless these factors are taken into consideration.

The COD test gives no indication of whether or not the waste is degradable biologically and nor does it indicate the rate at which biological oxidation would proceed and hence the rate at which the oxygen would be required in a biological system.

Despite all these limitations, the COD test continues to remain a very important parameter in management and design of the treatment plants because of its rapidly in determination. For all practical purposes its values are very close to the amount of chemically oxidizable carbonaceous matter which may be quite useful in the control of treatment processes. COD values cannot be corresponded with that of BOD values. In general, COD is more than the BOD values for most of the industrial wastes. COD values are taken as basis for calculation of the efficiency of the treatment plants and also figure in the standards for discharging industrial / domestic effluents in various kinds of waters.

PRINCIPLE

Chemical Oxygen Demand (COD) is the measure of Oxygen consumed during the oxidation of the oxidizable organic matter by a strong oxidizing agent. Potassium dichromate in the presence of sulphuric acid is generally used as an oxidizing agent in determination of COD.

The sample is refluxed with $K_2Cr_2O_7$ and H_2SO_4 in presence of mercuric sulphate to neutralize the effect of chlorides, and silver sulphate (catalyst). The excess of potassium dichromate is titrated against ferrous ammonium sulphate using ferroin as an indicator. The amount of $K_2Cr_2O_7$ used is proportional to the oxidizable organic matter present in the sample.

REAGENT

A. Potassium dichromate solution, 0.25 N

Dissolve 12.259 g of dried A.R. grade $K_2Cr_2O_7$ in distilled water to make 1 litre of solution.

B. Potassium dichromate solution, 0.025 N

Dilute 0.25 N $K_2Cr_2O_7$ 10 times. (100 → 1000 ml)

C. Ferrous ammonium sulphate, 0.1 N

Dissolve 39.2 g of Fe $(NH_4)_2$ $(SO_4)_2.6H_2O$ in water adding 20 ml conc. H_2SO_4 to make 1 litre of solution, standardize this solution with $K_2Cr_2O_7$. For standardization, dilute 10.0 ml of $K_2Cr_2O_7$ to about 100 ml, add 30 ml of conc. H_2SO_4 and titrate with ferrous ammonium sulphate using ferroin as an indicator.

D. Ferrous ammonium sulphate, 0.01N

Dissolve 0.1 N ferrous ammonium sulphate to 10 times (100 → 1000 ml).

E. Ferroin indicator

Dilute 1.485 g of 1, 10 – phenonthroline and 0.695 g of ferrous sulphate ($FeSO_4.7H_2O$) in distilled water to make 100 ml of solution.

F. Sulphuric acid

H_2SO_4, conc. (sp. Gr. 1.84)

G. Mercuric sulphate

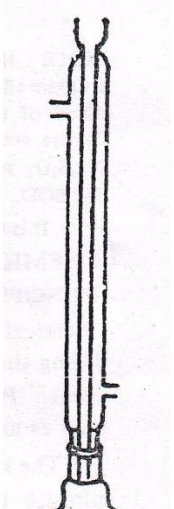
$HgSO_4$, solid

H. Silver sulphate

Ag_2SO_4, solid

PROCEDURE

1. Take 20 ml of sample in a 250 – 500 ml COD flask. (Round bottom or conical flask with a ground joint for Liebig reflux condensor.
2. If the sample is excepted to have COD more than 50 mg/L, add 10 ml of 0.25 N potassium dichromate solution (In case the cod is expected below 50 mg/L, add 10 ml of 0.025 N $K_2Cr_2O_7$). Extreme care should be taken in case of low COD samples. A small trace of organic matter in glassware may contribute a significant error.
3. Add a pinch of Ag_2SO_4 and $HgSO_4$. If the sample contains chlorides in higher amount, $HgSO_4$ is added in the ratio of 10 : 1, to the chlorides. COD cannot be determined accurately if the sample contains more than 2000 mg/L of chlorides.
4. Add 30 ml of sulphuric acid
5. Reflux at least for 2 hours on a water bath or a hot plate. The reflux assembly is shown in Fig. Remove the flasks, cool and add distilled water to make the final volume to about 140 ml.
6. Add 2 – 3 drops of ferroin indicator mix thoroughly and titrate with 0.1 N ferrous ammonium sulphate (with 0.01 N ferrous ammonium sulphate if 0.025 N $K_2Cr_2O_7$ has been used).
7. Run a blank with distilled water using same quantity of the chemicals.



CALCULATION

$$\text{COD, mg/L} = \frac{(b - a) \times N \text{ of Ferrous ammonium sulphate} \times 1000 \times 8}{\text{ml sample}}$$

Where, a = ml of titrant with sample

B = ml of titrant with blank.

Bacteriological Examination of Water

The Coliform MPN Test

Microorganism pathogenic to humans that are transmitted by water include bacteria (including blue – green algal toxins), viruses and protozoa. Most of the organisms transmitted by water usually grow in the intestinal tract of man and leave the body in the feces. Fecal pollution of water used for swimming and drinking can then occur resulting in transmission of infectious micro organisms. The significance of this was recognized at the turn of the century when filtration and disinfection of drinking water was begun in the USA. This results in the almost complete elimination of waterborne cholera and typhoid in the country.

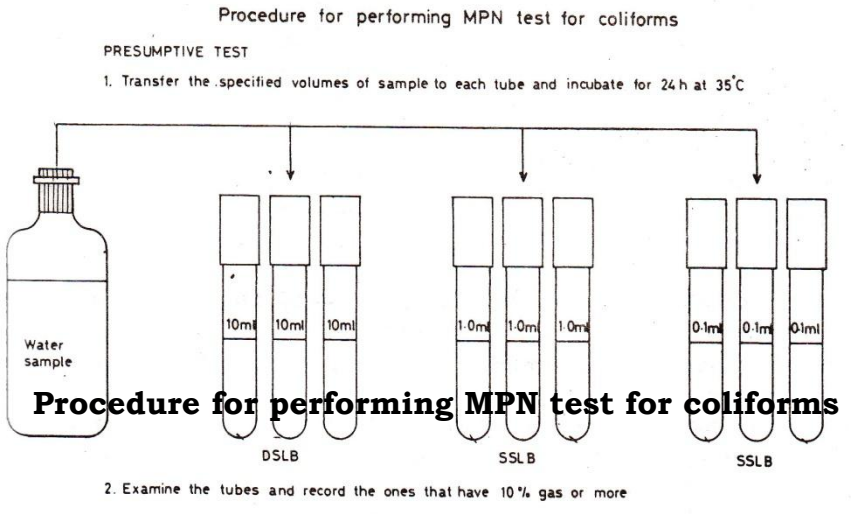
Routine examination of water for the presence of intestinal pathogens would be tedious and difficult, if not possible, task. It is much easier to demonstrate the presence of some of the non pathogenic intestinal types such as *Escherichia coli* and *Streptococcus jaecalis.* These organisms are always found in the intestines and normally are not present in soil or water : hence, when they are detected in water, it can be assumed that the water has been contaminated with fecal material.

Coliform bacteria (of which *E.coil* is a member) are often associated with enteric pathogenic organisms and have been shown to be useful indicators of the presence of fecal contamination.

Coliform bacteria occur normally in the intestines of humans and other warm – blooded animals and are discharged I great numbers in human and animal waste. In polluted water, coliform bacteria are found in densities roughly proportional to the degree of fecal pollution. When members of the coliform group are present, other kinds of microorganisms capable of causing disease also may be present.

Coliform bacteria are more hardy than disease – causing, non – spore – forming bacteria ; therefore, their absence from water is an indication that the water is bacteriologically safe human consumption. However, they are less sensitive than viruses and protozoan cysts to environmental factors (pH, temperature etc.,) and to disinfection. The presence of coliform bacteria, on the other hand, is an indication that disease – causing bacteria also may be present and that the water is unsafe to drink.

The coliform group includes all aerobic, facultatively anerobic, gram – negative, non – spore –forming, rod shaped bacteria which ferment lactose with gas production in prescribed culture media within 48 hours at 35^{o}C. Coliform bacteria include *E.coil, Citrobacter, Enterobacter and Klebsiella* species. An MPN (most probable number) test and the member filter test have been the methods most commonly used for the detection of coliforms in water. The membrane filter cannot be used easily with turbid waters because they will clog.

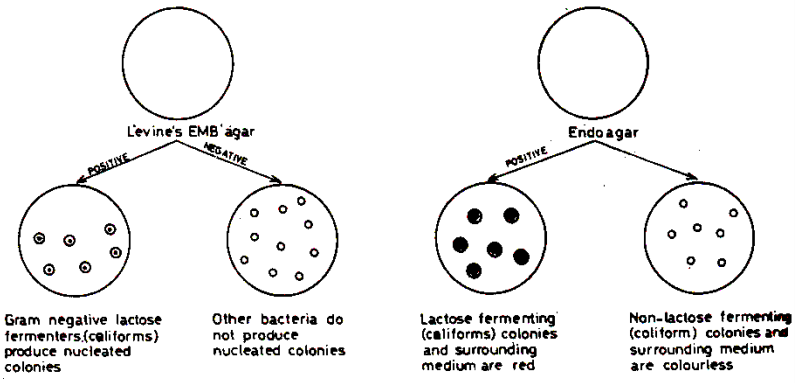


Procedure for performing MPN test for coliforms

The MPN test for coliforms consists of three steps: a presumptive test, a confirmed test and a complete test.

The first step is the presumption test. A set of tubes of lauryl sulfate tryptose (LST) lactose broth is inoculated with samples of water and incubated. Lauryl sulfate is a surface – active detergent which inhibits the growth of gram – positive organisms while encouraging the growth of coliforms. Coliforms use any oxygen present in the broth and then ferment in 24 or 48 hours is a positive test.

Once it is established that the gas – producing lactose fermenters are present in the water, it is presumed to be unsafe. However, gas formation may also be caused by non coloiform bacteria. Some of these, such as Clostridium perfringens, are gram positive. To confirm the presence of gram – positive lactose fermenters, the next step is to inoculate media such as Levine's eosin methylene blue (EMB) agar, Endo agar, or brilliant green lactose bile (BGLB) from positive presumptive tubes in what is called the confirmed test.



Confirmed Test

Levine's EMB agar contains methylene blue, which inhibits gram – positive bacteria. Gram – positive lactose fermenters (coloiforms) that grow on this medium will produce 'nucleated colonies' (dark centers). *E.coil* and *Enterbacter aerogenes* can be differentiated on the basis of size and the

presence of a greenish metallic sheen. *E.coil* cxolonies are small and have this metallic sheen, whereas, E.aerogenes colonies usually lack the sheen and are larger. Differentiation in this manner is not completely reliable, however, *E.coil* is the more reliable sewage indicator since it is not normally present in the soil, while E.aerogenes has been isolated from grains and soil.

Endo agar contains a sulfite indicator, which makes identification of lactose fermenters relatively easy. Coliform colonies and the surrounding medium appear red on endo agar. Non fermenters of lactose, on the other hand, are colourless and do not affect the colour of the medium. In addition to these two media, there are several other media that can be used for the confirmed test. Brilliant green bile lactose broth, Eijkman's medium and EC medium are a few others that can be used.

BGLB broth, in addition to containing lactose, also contains two components inhibitory to gram – positive bacteria. Brilliant green is a dye related to crystal violet and belongs to the triplhenyl methane dye series. Ox bile is a surface active agent which inhibits the growth of gram – positive bacteria.

Gas production in 24 or 48 hrs confirms the results of the presumptive step. The number of coliforms per 100mL of water is then calculated from the distribution of positive and negative tubes in the test by referring to an appropriate table. Results are reported as coliform MPN per 100 mL of water.

In some cases the organisms must be isolated and stained to provide the completed test. Positive BGLB tubes are streaked on eosin – methylene blue (EMB) agar. The two dyes. Eosin and methylene blue, also inhibit the growth of gram positive organisms. Typical colonies are isolated on nutrient agar slants and inoculated into LST broth. If gas is now formed by 24 or 48 hours, a gram stain is made from the growth on the slant. If the cells are gram negative after

examination under oil and there is no indication of spores, the completed test is considers to be positive. Further bio chemical studies (IMViC) may be performed on isolated cultures. In practice the completed test is seldom performed.

All three test are necessary to prove that an organism in a water sample is in truth a coliform. In actual practice, when it has been shown that the presumptive and confirmed tests give essentially the same result, then the completed test is generally not done because of the time it takes.

PROCEDURE

First Period

Materials

Water sample

3 Durham tubes of Double Strength Broth

6 Durham tubes of single Strength Lactose Broth

10 ml Pipette and 1 ml Pipette

Incubator at 35^0C

Presumptive test

1. Set up three DSLB and six SSLB tubes. Label each tube according to the amount of water that is to be dispended to it: 10ml, 1.0ml and 0.1ml respectively.
2. Mix the bottle of water to be tested by shaking.
3. With a 10ml. pipette transfer 10ml of water to the DSLB tubes.
4. With a 1.0ml pipette, transfer 1.0ml of water to each of the middle set of SSLB tubes and 0.1ml to each of the last three SSLB tubes.
5. Inoculate the tubes at 35^oC for 48h.

Second Period

Materials

Incubate tubes from the previous week

Petriplate of Levine's EMB agar

Petriplate of Endo agar

Inoculation loop and gas burner

Presumptive Test

6. Examine the tubes and record the number of tubes in each set that have 10% gas or more. Determine the MPN by referring the table.

Confirmed Test

7. Select one positive lactose broth tube from the presumptive test and streak one plate of each of Levine's EMB agar and Endo agar. Use a streak method which will produce good isolation of colonies.
8. Incubate the plate for 24h at 35^{o}C.

Third Period

Materials

Incubated plates from the previous week.

Confirmed Test

9. Look for typical colonies on both kinds of media. If no coliforms are present, the water is considered to be safe to drink.

MPN TABLE

10ml	1ml	0.1ml	MPN/100ml	10ml	1ml	0.1ml	MPN/100ml
0	0	0	3	2	0	0	9.1
0	1	0	3	2	0	1	14
0	0	2	6	2	0	2	20
0	0	3	9	2	0	3	26
0	1	0	3	2	1	0	15

10ml	1ml	0.1ml	MPN/100ml	10ml	1ml	0.1ml	MPN/100ml
0	1	1	6.1	2	1	1	20
0	1	2	9.2	2	1	2	27
0	1	3	12	2	1	3	34
0	2	0	6.2	2	2	0	21
0	2	1	9.3	2	2	1	28
0	2	2	12	2	2	2	35
0	2	3	16	2	2	3	42
0	3	0	9.4	2	3	0	29
0	3	1	13	2	3	1	36
0	3	2	16	2	3	2	44
0	3	3	19	2	3	3	53
1	0	0	3.6	3	0	0	23
1	0	1	7.2	3	0	1	39
1	0	2	11	3	0	2	64
1	0	3	15	3	0	3	95
1	1	0	7.3	3	1	0	43
1	1	1	11	3	1	1	75
1	1	2	15	3	1	2	120
1	1	3	9	3	1	3	160
1	2	0	11	3	2	0	93
1	2	1	15	3	2	1	150
1	2	2	20	3	2	2	210
1	2	3	24	3	2	3	290
1	3	0	16	3	3	0	240
1	3	1	20	3	3	1	460
1	3	2	24	3	3	2	1100
1	3	3	29	-	-	-	-

Media Composition

Lactose broth			**(g / l)**
	Beef extract	:	6.0
	Peptone	:	10.0
	Lactose	:	10.0
Eosin Methylene Blue agar medium			**(g / l)**
	Peptone	:	10.0
	Lactose	:	5.0
	Sucrose	:	5.0
	Dipotassium	:	2.0
	Hydrogen phosphate		
	Eosin	:	4.0
	Methylene blue	:	0.06
	Agar	:	15.0
Endo agar medium			**(g / l)**
	Peptone	:	10.0
	Lactose	:	10.0
	Dipotassium	:	6.5
	Hydrogen phosphate		
	Sodium sulphate	:	2.5
	Basic fuchsin dye	:	0.5
	Agar	:	15.0

SECONDARY STANDARDS FOR DRINKING WATER

Contaminant	Level
Chloride	250 mg/L
Colour	15 colour units
Copper	1 mg/L
Corrosivity	Non corrosive
Foaming agents	0.5 mg/L
Iron	0.3 mg/L
Manganese	0.05 mg/L
Odour	3 threshold odour number
pH	6.5 – 8.5
Sulfate	25 mg/L
Total dissolved	
Solids	500 mg/L
Zinc	5 mg/L

MAXIMUM CONTAMINATION LEVELS (MCLS) FOR CERTAIN ORGANIC CHEMICALS

Contaminant	Principle health effects	MCL (mg/L)
Endrin	Nervous system / kidney effects	0.002
Lindane	Nervous system / kidney effects	0.004
Methoxyxhlor	Nervous system / kidney effects	0.10
2,4 – D	Liver / kidney effects	0.10
2,4,5 – TP	Liver / kidney effects	0.01

Silvex		
Toxaphane	Cancer risk	0.005
Trihalo methanes	Cancer risk	0.10

MAXIMUM CONTAMINATION LEVELS (MCLS) FOR CERTAIN INORGANIC CHEMICALS

Contaminant	**Principle health effects**	**MCL (mg/L)**
Arsenic	Dermal and nervous system toxicity effects	0.05
Barium	Circulatory system effects	1.0
Cadmium	Kidney effects	0.010
Chromium	Liver / Kidney effects	0.05
Fluride	Skeletal damage	1.8 at 20^{o}C
Lead	Central and peripheral nervous system, kidney effects, highly toxic to infants and pregnant women	0.005
Mercury	Central nervous system disorder, kidney effects	0.002
Nitrate and Nitrite	Methemoglobinemia (blue baby syndrome)	10 (as N)
Selenium	Gastrointestinal effects	0.01
Silver	Skin discolouration (argyria)	0.05