A MANUAL ON WATER QUALITY ANALYSIS

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WATER QUALITY MONITORING

The following are common reasons to do water quality testing at various levels: to ensure safe drinking water, to identify problems, to adopt precautionary measures, to raise awareness, to determine the effectiveness of the WTS process, to select an appropriate water source, to influence government to supply safe water.

Water quality monitoring helps communities establish sound scientific practices to measure the health of local water body so stakeholders can take actions to protect and improve desired conditions and uses of the river and its watershed for the future.

Water is one of the most abundant and important substances on Earth. Water comprises over 70 percent of the Earth's surface and 50 to 80 percent of every living organism's weight—it truly connects all living things. Each of us – student, bird, farmer and plant – depends on the same global, water centered system. Within this system, our lives depend on the small volume of fresh water. Fresh, clean, drinkable water constitutes only one half of one percent of all the Earth's water. We depend on our streams and rivers to deliver much of this drinking water, as well as provide for irrigation for agriculture, recreation and other uses. Many animals and plants could not live without clean river water. Humans are the only species with the ability to manage water resources. With this ability comes an important responsibility to understand and protect water body and rivers. A vital tool for such action is water quality monitoring. The information that we collect helps us:

- Determine the overall health of our water body
- Understand our water body and their role in the watershed
- Make good watershed management decisions
- Identify specific water quality problems
- Most importantly, take wise action to improve or protect the water quality of our water body

Drinking Water Quality

We find our drinking water from different places depending on where we live in the world. Three sources that are used to collect drinking water are:

1. Ground water – Water that fills the spaces between rocks and soil making an aquifer. Ground water depth and quality varies from place to place. About half of the world's drinking water comes from the ground.

2. Surface water **-** Water that is taken directly from a stream, river, lake, pond, spring or similar source. Surface water quality is generally unsafe to drink without treatment.

3. Rainwater **-** Water that is collected and stored using a roof top, ground surface or rock catchment. The quality of rain water collected from a roof surface is usually better than a ground surface or rock catchment.

Water is in continuous movement on, above and below the surface of the earth. As water is recycled through the earth, it picks up many things along its path. Water quality will vary from place to place, with the seasons, and with various kinds of rock and soil which it moves through. For the most part, it is natural processes that affect water quality. For instance, water moving through underground rocks and soils may pick up natural contaminants, even with no human activity or pollution in the area. In addition to nature's influence, water is also polluted by human activities, such as open defecation, dumping garbage, poor agricultural practices, and chemical spills at industrial sites. Even though water may be clear, it does not necessarily mean that it is safe for us to drink. It is important for us to judge the safety of water by taking the following three qualities into consideration:

1. Microbiological – bacteria, viruses, protozoa, and worms
2. Chemical – minerals, metals and chemicals
3. Physical – temperature, colour, smell, taste and turbidity

Safe drinking water should have the following microbiological, chemical and physical qualities: Free of pathogens, Low in concentrations of toxic chemicals, Clear, Tasteless and colourless (for aesthetic purposes). When considering drinking water quality, in most cases microbiological contamination is the main concern since it is responsible for the majority of illnesses and deaths related to drinking unsafe water.

Water sampling

It is well recognized that the result of any test procedure can be no better than the sample on which it is performed. The objective of sampling is to collect a portion of material small enough in volume which can be transported easily and handed in the laboratory while still accurately representing the material being collected.

The sampling programme defines the portion of the whole to which rest results apply. The variations of the whole material with respect to time, area, depth, and occasionally, rate of flow must be taken into consideration to ensure representative nature of the sample. It is not possible to specify detailed procedure for collection of all types of samples. However, a general guidelines as suggested below may be followed:

1. While collecting a sample from the distribution system, flush lines adequately, taking into consideration the diameter and length of the pipe to be flushed and the velocity of flow.
2. Collect samples from tube-wells only after sufficient pumping to ensure that the sample represents the ground water source.

3. When samples are to be collected from a river or stream, analytical results may vary with depth, flow, distance from the shore and from one shore to the other. If equipments are available for collection of "integrated" sample from top to be bottom in the middle of the stream and composite the sample according to the flow, Grab or catch sample should be collected in the middle of the stream and at mid depth.

4. Since lakes and reservoirs are generally undergo considerable variation due to normal natural causes, choose location, depth and frequency of sampling depending on local conditions and the objective of the study.

5. Make detailed record of every sample collected and identify each contained by attaching a tag or label. Record information like date, time and exact location, weather condition, stream-flow etc.

6. Normally a 2-litre sample would be sufficient for most physical and chemical tests. Never use the sample for chemical, bacteriological and microscopic examinations, because collection and test procedures for the latter tests are different.

7. The interval between collection and analysis of the sample should be shortest possible.

Recording and Reporting of Data

1. All the Laboratory shall keep records of submitted samples and completed analysis in a manner that provides for the data retrievability, the sample preservation and the persons responsible for the sampling and analysis. All laboratory data sheets shall be dated and signed by the analysts and the Head of the Laboratory or his designee.

2. Electrical conductivity, pH, temperature, and turbidity should be recorded in units specified for the respective tests and the results of other chemical examinations shall be expressed in terms of substances or ions actually determined and reported as milligram per litre, which may be considered for all practical purposes as equivalent to parts per million (ppm).

3. A commonly used and functional method of recording laboratory data is a note book specifically printed for this purpose. The pages are serially numbered in pairs with a carbon between the pages to provide matching serial numbered copy of the data. These books are permanently bound but the duplicate page is performated for easy removal. The duplicate page may then be filed in a system wherefrom it may be readily retrieved. The pages of the note book are generally lined in a grid pattern with provision for such information as project identification, data, reference to the analytical procedure, the observations, sample calculations, as mg/1 and signature of the analyst.

Procedure for Analysis of Various Parameters

I. Physical Parameters

A. Colour

The platinum cobalt method of measuring colour is the standard method, the unit of colour being that produced by 1 mg platinum/ litre in the form of chloroplatinate ion. It is applicable to measure the colour of potable water and of water in which colour is due to naturally occurring materials. Even a slight turbidity effects the colour. Remove turbidity by centrifugation for 1 hours. As colour varies with pH, specify the pH at which colour is measures.

Field Method: As the platinum-cobalt standard method is not convenient for field use, compare water colour with that of glass discs held at the end of metallic tubes containing glass comparator tubes filled with sample and colourless distilled water. Match sample colour with the colour of the tube of clear water plus the caliberated coloured glass when viewed by looking towards a white surface. Collect representative samples in clean glassware and make the colour determination within a reasonable period.

Appratus: (a) Nessler tubes, 50 ml, tall form, (b) pH – meter

Preparation of standards: Dissolve 1.246 g potassium chloroplatinate, K_2PtCl_6 (equivalent to 500 mg metallic Pt) and 1.00 g. crystallized $CoCl_2$ 2O (equivalent to about 250 mg metallic Co) in distilled water with 100 ml conc. HCl and dilute to 1000 ml distilled water. This stock standard has a colour of 500 units. Prepare standards having colours of 5, 10, 15, 20,…….70 by diluting the stock colour standard with distilled water to 50 ml in Nessler tubes.

Procedure: Observe sample colour by filling a matched Nessler tube, to the ml mark with sample and comparing it with standards. Looking vertically downwards through tubes towards a while surface placed at such an angle that light is reflected upward through the columns of liquid. If turbidity is present, report as apparent colour. If value exceeds 70, dilute sample according so that value is within 70.

Measure pH of the sample: Report nearest to 1 if value is 1-50, nearest to 5 for 51-100, to 10 for 101-250 & to 20 for 251-500.

B. pH

The pH value of water, on a scale of 0 to 14, measures the concentration of hydrogen ions. The pH represents the balance between hydrogen ions and hydroxide ions in water. Solutions with more hydrogen than hydroxide ions have a pH value lower than 7 and are said to be acidic. Solutions with pH values higher than 7 have more hydroxide than hydrogen ions and are said to be basic, or alkaline. Pure distilled water is considered neutral, with a pH reading of 7. Water is basic if the pH is greater than 7; water with pH of less than 7 is considered acidic. For every one unit change in pH there is approximately a ten-fold change in how acid or basic the sample is. This means that each step on the scale represents a ten-fold change in the hydrogen concentration. For example, water

with a pH of 5 has ten times the number of hydrogen ions than water with a pH of 6 and is ten times more acidic. The pH for drinking water generally lies between 6.5 and 8.0.

Theory: The pH of a solution denotes the intensity of the acidity or alkalinity of a solution and is defined by the relationship:

$$pH + -\log_{10} CH$$

Where CH = the concentration of Hydrogen ions in gm. ion per litre.

The pH scale runs from 0.0 to 14 with 7.0 being neutral. Many surface water have pH between 6.0 to 8.5. The pH can be measured by the indicator method and by the electerometric method using a pH meter. The equipments needed include colour standards covering the pH range desired, indicator solutions corresponding to the colour standards, a comparator designed to facilitate the matching of the colour and special test tubes to hold the test sample. The electrometric method requires a pH metere which is a sensitive instrument.

Procedure: In this method the pH metere is first calibrated against a solution of known pH and the test well is then filled with the sample. The pH is read directly either from the scale on the instrument of digital display as the case may be. This method is most accurate and almost free from interferences. It is imperative to follow strictly the manufacturer's direction.

C. Turbidity

Suspension of particles in water interfering with passage of light is called turbidity. Turbid waters are undesirable from aesthetic point of view in drinking water supplies and may also affect products in industries. Turbid water also possess a number of problems in water treatment plants. Turbidity is measured to evaluate the performance of water treatment plants.

Principle: The method is based on a comparison of the intensity of light scattered by a sample and a standard reference under the same conditions. Higher the intensity of scattered light higher the turbidity.

Interference: Colour is the main source of interference the measurement of turbidity.

Appratus: Turbidimeter or Klett Summerson.

Reagents

1. Solution I: Dissolve 1.0 g hydrazine sulfats and dilute to 100 ml.
2. Solution II: Dissolve 10.g hexamethylene-tetramine and dilute to 100 ml.
3. Mix 5 ml of I with 5 ml of II. Allow to stand for 24 hrs. and dilute to 1000 ml. This will have turbidity of 400 units.
4. Standard turbidity suspension: Dilute 100 ml of solution III as prepared above to 100 ml too have turbidity of 40 units.

Procedure

1. Prepare calibration curves in the rage 0-400 unit by carrying out appropriate dilutions of solutions III and IV above and taking readings on turbidimeter.

2. Take sample or a suitably diluted aliquot and determine its turbidity either by visual comparison with the diluted standards or by reading on turbidimeter.
3. Read turbidity from the standard curves and apply correction due to dilution, if necessary.
4. Report the readings in turbidity unit.

D. Electrical Conductivity

Conductivity is a numerical expression of the ability of an aqueous solution to carry an electric current. This ability depends upon the presence of ions, their total concentration, mobility, valence and relative concentrations and on the temperature of measurement.

Apparatus

1. A good conductivity meter with cell.
2. Thermometer

Reagents

1. Conductivity water with conductivity less than 1 mho/ cm.
2. Standard potassium chloride solution (0.01 N). Dissolve 745.6 mg anhydrons KC1 in conductivity water and dilute to 1000 ml at 25oC. This has a conductivity of 1413 mhos/ cm at 25oC.

Procedure

Rinse conductivity cell with at least three portions of 0.01 N KC1 solution. Adjust temperature of a fourth portion to 25.0 ± 0.1° C. Measure conductivity which should be 1413 mhos/ cm. If not adjust the meter to read this value using the cell constant knob. Note the cell constant. Rinse cell with one or more portions of sample. Adjust temperature of a final portion to 25.0 ± 0.1° C. Read the conductivity value and note the temperature.

E. Total Dissolved Solids (TDS)

Total dissolved solids (TDS) are made up of inorganic salts (mainly sodium chloride, calcium, magnesium, and potassium) and small amounts of organic matter that are dissolved in water. There are areas of the world that have naturally high amounts of TDS in their drinking water. TDS in drinking water comes from natural sources, sewage, urban runoff and industrial wastewater. Brackish or saline aquifers can exist naturally or develop overtime in coastal regions with sea water infiltration due to lowering of aquifer depths. Drinking water with high concentrations of total dissolved solids will not make people sick. Although there are no direct health concerns, TDS concentrations greater than 1,200 mg/L (e.g. brackish or saline water) cause a bitter or salty taste. Some people can taste salt in drinking water at levels around 500 mg/L, and it may cause them to not use it and choose another, possibly contaminated, water source instead. Water with extremely low TDS concentrations (e.g. rainwater) may also be unacceptable because of its flat taste.

As mentioned earlier Electrical conductivity (EC) of a substance is defined as its ability to conduct or transmit electricity. The presence of chemicals (such as calcium and magnesium ions) gives water

the ability to conduct electricity. Testing for EC does not give specific information about the chemicals present in water, but it gives an estimation of TDS. Thus, the EC of water is an indirect measure of dissolved chemicals.

$$TDS\ (mg/L\ or\ ppm) = EC\ (\mu S/cm) \times 0.67$$

II. Chemical Parameters

A. Total Alkalinity

Principle: Alkalinity of sample can be estimated by titrating with standard Hydrochloric acid (0.02N) at room temperature using methyl orange indicator. Titration to sharp change from yellow to orange of methyl orange indicator will indicate total alkalinity (complete neutralisation of OH^-, CO_3^{--}, HCO_3^-).

Apparatus

a. Beakers: The size and form will depend upon the electrode and the size of the sample to be used for determination of alkalinity.

b. Pipettes (volumetric)

c. Flasks (volumetric): 1000mL, 200mL, 100mL

Reagents and standards

a. Stock HCl: Prepare 1 N HCl from laboratory reagent.

b. Standard HCl, 0.02 N: Prepare 0.1N HCl by diluting conc HCl to 1000mL.

c. Methyl orange indicator: Dissolve 0.5g and dilute to 1000mL with CO2 free distilled water (pH 4.3-4.5).

B. Total Acidity

Principle: Acidity of sample can be estimated by titrating with standard Sodium hydroxide (0.02N) at room temperature using phenolphthalein indicator. Titration to sharp change from colorless to pink will indicate total acidity.

Apparatus

a. Beakers: The size and form will depend upon the electrode and the size of the sample to be used for determination of alkalinity.

b. Pipettes (volumetric)

c. Flasks (volumetric): 1000mL, 200mL, 100mL

Reagents and standards

a. Stock NaOH: Prepare 1 N NaOH (4 gms in 100 ml) from laboratory reagent.

b. Standard HCl, 0.02 N: Prepare 0.1N HCl by diluting conc NaOH to 1000mL.

c. Phenolphthalein indicator: Dissolved 0.5g in 500mL 95% ethyl alcohol. Add 500mL distilled water. Add dropwise 0.02N NaOH till faint pink colour appears (pH 8.3).

C. Hardness

Hardness in water causes scale formation in boilers. It is also objectionable from view point of water use for laundry and domestic purposes since it consumes a large quantity of soap. Generally, salts of

Ca and Mg contribute hardness to natural waters. Hardness may be classified either as (1) carbonate and non-carbonate hardness or (2) calcium and magnesium hardness or (3) temporary and permanent hardness.

Principle

In alkaline condition EDTA reacts with Ca and Mg to form a soluble chelated complex. Ca and Mg ions develop wine red colour with Eriochrome Black T under alkaline condition. When EDTA is added as a titrant the Ca and Mg divalent ions get complexed resulting in sharp change from wine read to blue which indicates end point of the fitration. The pH for this titration has to be maintained at 10.0 ± 0.1. At a higher pH, i.e., about 12.0 Mg ion precipitates and only Ca ion remains in solution. At this pH Murex indicator form a pink colour with Ca^{++} When Edta is added Ca^{++} gets complexed resulting in a change from pink to purple which indicates end point of the reaction.

Interference

Metal ions do interfere but lkexan be overcome by addition of inhibitors.

Reagents

1. Buffer solution: Dissolve 16.9 g NH_4Cl in 143 NH_4OH. Add 1.25 g EDTA Mg salt to obtain sharp change in indicator and dilute to 250 ml. This has to be titrated with standard calcium solution to avoid interference produced by addition of EDAT to the buffer.
2. Inhibitor: Dissolve 4.5 g hydroxyl amine hydrochloride in 100 ml 95% ethyl alcohol.
3. Erichrome black T Indicator: Mix 0.5 dye with 100g NaCl to prepare dry powder.
4. Murex indicator: Dry powder.
5. Sodium hydroxide 2N: dissolve 80 g NaOh and dilute to 1000 ml.
6. Standard EDTA solution 0.01 M: Dissolve 3.723 g EDTA sodium salt and dilute to 1000 ml. Standardize against standard Ca solution, 1 ml = 1 mg $CaCO_3$. **Procedure**

a. Total Harness

1. Take 25 or 50 ml well mixed sample in porcelain dish or conical flask.
2. Add 1-2 ml buffer solution followed by 1 ml inhibitor.
3. Add a pinch of Erichrome black T and titrate with standard EDTA (0.01 M) till wine red colour changes to blue. Note down to vol. Of EDTA required. (A)
4. Run a reagent blank if buffer is not checked properly.
5. Calculate vol. of EDTA required by sampe, C= (A-B) from vol. of EDTA required in steps 3 & 4.

b. Calcium Hardness

1. Take 25 or 30 ml sample in a porcelain dish.
2. Add 1 ml NaOH to raise pH to 12.0 and a pinch of indicator.
3. Titrate with EDTA till pink colour changes to purple. Note the vol. of EDTA used (A').

c. Magnesium Harness

Magnesium hardness = Total hardness – Ca hardness.

D. Chloride

The chloride estimation has two different purposes.

1. If the test is done regularly on a water supply and there is a sudden increase, it may indicate pollution due to ingress of sewerage or other chloride rich wastes.

2. Many ground waters have chloride content high enough to be of objectionable taste. By using the chloride test, the well with the lowest amount of chloride can be identified. If several well are being pumped, it can be planned in such a way that the lowest chloride content is obtained.

Principle

The Mohr method for the determination of chloride in water is based upon the fact that in solution containing chloride and chromate, silver reacts with all the chloride and precipitates before the reaction with chromate begins. The appearance of the brick-red colour of the silver chromate precipitate is the end-point of the titration.

Reagents

1. Potassium chromate indicator, 5% solution (dissolve 5 gm Potassium chromate in 100 ml distilled water).

2. Standard silver nitrate ($AgNO_3$) solution 0.0282 N (1 ml = 1 mg of chloride).

Procedure

1. Take 100 ml of sample and adjust the pH between 7.0 and 8.0

2. Take 50 ml of well mixed sample adjusted to pH 7.0 – 8.0 and add 1 ml of potassium chromate indicator.

3. Titrate with standard silver nitrate solution till the colour changes from yellow to brick-red.

4. Titrate 50 ml of distilled water in the same way after adding 1 ml of potassium chromate indicator to establish reagent blank.

E. Dissolved Oxygen (DO)

Dissolved oxygen refers to the amount of oxygen (O) dissolved in water. Because fish and other aquatic organisms cannot survive without oxygen, dissolved oxygen is one of the most important water quality parameters. Dissolved oxygen is usually expressed as a

concentration of oxygen in a volume of water (milligrams of oxygen per liter of water, or mg/L). In nature, oxygen gets into water in two ways. Oxygen from the atmosphere is mixed into (diffuses into) the water from the atmosphere. Where water is rough (for example, where water is tumbling over rocks or where there are waves), the oxygen and the water mix more easily, resulting in more oxygen from the atmosphere being dissolved in the water. Oxygen is also introduced into water by green aquatic plants and algae during photosynthesis. Cold water holds more oxygen than warm water. For example, pure water at 4°C (just above

freezing) can hold about 13.2 mg/L dissolved oxygen at 100 percent saturation, while pure water at 250°C can hold only 8.4 mg/L at 100 percent saturation. Water with a high concentration of dissolved minerals cannot hold as much dissolved oxygen as pure water.

The Winkler method with azide modification

Principle

Oxygen present in sample rapidly oxidises the dispersed divalent manganous hydroxide to its higher valency, which is precipitated as a brown hydrated oxide after the addition of NaOH/KOH and Kl. Upon acidification, manganese reverts to divalent state and liberates iodine from Kl equivalent to the original DO content. The liberated iodine is titrated against $Na_2S_2O_3$ (N/40) using starch as an indicator.

Apparatus and equipment

a. BOD bottles, capacity 300mL

b. Sampling device for collection of samples

Reagents

a. Manganese sulphate: Dissolve 480g $MnSO_4.4H_2O$ or 400g $MnSO_4.2H_2O$ in distilled to 1000mL. Filter if necessary. This solution should not give colour with starch when added to an acidified solution of Kl.

b. Alkali iodide-azide reagent.

1. For saturated or less than saturated samples: Dissolve 500g NaOH (or 700g KOH) and 150g Kl (or 135g Nal) in distilled water and dilute to 1000mL. Add 10g sodium azide, NaN_3 dissolved in 40mL distilled water. This solution should not give colour with starch solution when diluted and acidified.

2. For supersaturated samples: Dissolve 10g NaN_3 in 500mL distilled water. Add 480g NaOH and 750g Nal and stir to dissolve the contents.

Cautions: Do not acidify this solution because toxic hydrozoic acid fumes may be produced.

c. Sulphuric acid: H_2SO_4, conc., 1mL is equivalent to about 3mL alkali-iodide-azide reagent.

d. Starch indicator: Prepare paste or solution of 2.0g of soluble starch powder and 0.2g salicylic acid as preservative in distilled water. Pour this solution in 100mL boiling distilled water. Continue boiling for a few minutes, cool and then use.

e. Stock sodium thiosulphate, 0.1N: Dissolve 24.82g $Na_2S_2O_3.5H_2O$ in distilled water. Preserve by adding 0.4g solid NaOH or 1.5mL of 6 N NaOH and dilute to 1000mL.

Sample collection, preservation and storage

Sampling for dissolved oxygen depends upon the source and method of analysis. While sampling, sample should not remain in contact with air or should not be agitated. These conditions can cause severe change in gaseous content. Sampling from any depth in streams, lakes or reservoir needs special precautions to eliminate changes in pressure and temperature. There are specific procedures and equipment developed for sampling water under pressure and unconfined water. Sample should

be collected in narrow mouth glass BOD bottles of 300mL capacity. Let the bottle overflow for some time and then stopper the bottle so that no air bubbles could form. The DO determination should be carried out immediately after sampling.

Procedure

1. Collect sample in a BOD bottle using Do sampler.
2. Add 1mL $MnSO_4$ followed by 1mL of alkali-iodide-azide reagent to a sample collected in 250 to 300mL bottle up to the brim. The tip of the pipette should be below the liquid level while adding these reagents. Stopper immediately. Rinse the pipettes before putting them to reagent bottles.
3. Mix well by inverting the bottle 2-3 times and allow the precipitate to settle leaving 150mL clear supernatant. The precipitate is white if the sample is devoid of oxygen, and becomes increasingly brown with rising oxygen content.
4. At this stage, add 1mL conc. H_2SO_4. Replace the stopper and mix well till precipitate goes into solution.
5. Take 201mL of this solution in a conical flask and titrate against standard $Na_2S_2O_3$ solution using starch (2mL) as an indicator. When 1mL $MnSO_4$ followed by 1mL alkali-iodide-azide reagent is added to the samples as in (2) above, 2mL of original sample is lost. Therefore 201mL is taken for titration which will correspond to 200mL of original sample.

   200 300/ (300-1) = 201mL

F. Biochemical Oxygen Demand (BOD)

The Biochemical Oxygen Demand (BOD) is an empirical standardized laboratory test which measures oxygen requirement for aerobic oxidation of decomposable organic matter and certain inorganic materials in water, polluted waters and wastewater under controlled conditions of temperature and incubation period. The quantity of oxygen required for above oxidation processes is a measure of the test. The test is applied for fresh water sources (rivers, lakes), wastewater (domestic, industrial), polluted receiving water bodies, marine water (estuaries, coastal water) and also for finding out the level of pollution, assimilative capacity of water body and also performance of waste treatment plants.

Principle

This test measures the oxygen utilised for the biochemical degradation of organic material (carbonaceous demand) and oxidation of inorganic material such as sulphides and ferrous ions during a specified incubation period. It also measures the oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. Temperature effects are held constant by performing a test at fixed temperature. The methodology of BOD test is to compute a difference between initial and final Do of the samples incubation. Minimum 1.5 L of sample is required for the test. DO is estimate by iodometric titration. Since the test is mainly a bio-assay

procedure, it is necessary to provide standard conditions of temperature, nutrient supply, pH (6.5-7.5), adequate population of microorganisms and absence of microbial-growth-inhibiting substances. The low solubility of oxygen in water necessitates strong wastes to be diluted to ensure that the demand does not increase the available oxygen. A mixed group of microorganisms should be present in the sample; otherwise, the sample has to be seeded. Generally, temperature is controlled at 20ºC and the test is conducted for 5 days, as 70 to 80% of the carbonaceous wastes are oxidized during this period. The test can be performed at any other temperature provided the correlation between BOD_5 20ºC is established under same experimental condition (for example BOD_5, 27ºC) is equivalent to BOD_3, 27ºC) for Indian conditions. While reporting the results, the incubation period in days and temperature in ºC is essential to be mentioned.

Equipment and apparatus

a. BOD bottles 300mL capacity (clean with a detergent, rinse thoroughly and drain before use) with a water seal.
b. Incubator or water-bath to be controlled at 20ºC or at any desired temperature 1ºC. Exclude all light to prevent photosynthetic production of DO.

Reagents and standards

All reagents listed in DO estimation are used for BOD. In addition following reagents are required:

a. Phosphate buffer: Dissolve 8.5g KH_2PO_4, 21.75g K_2HPO_4, 33.5g $Na_2HPO_4.7H_2O$ and 1.7g NH_4C; in distilled water and dilute to 1000mL. The pH should be 7.2without further adjustment. Discard reagent if there is any sign of biological growth.
b. Magnesium sulphate: Dissolve 22.5g $MgSO_4.7H_2O$ in about 700mL of distilled water and dilute to 1 Litre.
c. Calcium chloride: Dissolve 27.5g anhydrous $CaCl_2$ in about 7000mL of distilled water and dilute to 1 Litre.
d. Ferric chloride: Dissolve 0.25g $FeCl_3.6H_2O$ in about 700mL of distilled water and dilute to 1 L.
e. Sodium sulphate solution 0.025N: Dissolve 1.575g Na_2SO_3 in distilled water and dilute to 1000mL. Solution should be prepared daily.
f. Acid and Alkali solutions 1N: Prepare 1N H_2SO_4 and 1N NaOH or neutralization of caustic or acidic sample
g. Nitrification inhibitor: 2-chloro-6-(trochloromethyl) pyridine [Nitrification inhibitor 2570-24 (2.2% TCMP), Hach Co. equivalent]
h. Glucose-glutamic acid solution: Dry reagent grade glucose and glutamic acid at 103ºC for 1h. Dissolve 150 mg glucose and 150mg glucose acid in distilled water and dilute to 1000mL. Prepare fresh immediately before use.

Sample collection, preservation and storage

Grab or composite samples are collected. Keep composite samples at or below 4ºC during compositing. Samples for BOD may degrade significantly during storage. Minimise reduction of BOD by analyzing samples promptly or by cooling it to near freezing temperature during storage. The maximum holding time recommended between collection and analysis is 48 hours. Warm chilled samples to 20-27ºC ± 3ºC before analysis. State storage time and condition as part of results.

Procedure

Preparation of dilution water:

a. The source of dilution water may be distilled water, tap or receiving-stream water free of biodegradable organics and bioinhibitory substances such as chlorine or heavy metals.
b. Aerate the required volume of dilution water in a suitable bottle by bubbling clean-filtered compressed air for sufficient time to attain DO saturation at room temperature or at 20ºC/27ºC. Before use stabilise the water at 20ºC/27ºC.
c. Add 1mL each of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride solutions in that order for each Litre of dilution water. Mix well. Quality of dilution water may be checked by incubating a BOD bottle full of dilution water for 5 days at 20ºC for 3 days at 27ºC. DO uptake of dilution water should not be more than 0.2mg/L and preferable not more than 0.1mg/L.
d. For wastes which are not expected to have sufficient microbial population, seed is essential. Preferred seed is effluent from a biological treatment system. Where this is not available, supernatant from domestic wastewater (domestic sewage) settled at room temperature for at least 1h but not longer than 36hours is considered sufficient in the proportion 1-2mL/L of dilution water. Adopted microbial population can be obtained from the receiving water microbial population can be obtained from the receiving water body preferably 3-8 km below the point of discharge. In the absence of such situation, develop an adapted seed in the laboratory.
e. Determine BOD of the seeding material. This is seed control. From the value of seed control determine seed DO uptake. The DO uptake of seeded dilution water should be between 0.6mg/L and 1mg/L.

Sample preparation:

a. Neutralise the sample to pH 7, if it is highly acidic or alkaline.
b. The sample should be free from residual chlorine. If it contains residual chlorine remove it by using $Na_2S_2O_3$ solution as described below.
c. Take 50mL of the sample and acidify with addition of 10mL 1 + 1 acetic acid. Add about 1g Kl. Titrate with 0.025N $Na_2S_2O_3$, using starch indicator. Calculate the volume of $Na_2S_2O_3$ required per Litre of the sample and accordingly add to the sample to be tested for BOD.

d. Certain industrial wastes contain toxic metals, e.g. planting wastes. Such samples often require special study and treatment.

e. Bring samples to 20 ± 1°C before making dilutions.

f. If nitrification inhibition is desired, add 3mg 2-chloro-6-(trichloromethyl) pyridine (TCMP) to each 300mL bottle before capping or add sufficient amount to the dilution water to make a final concentration of 30mg/L. Note the use of nitrogen inhibition in reporting results.

g. Samples having high DO contents, DO ≥ 9mg/L should be treated to reduce the DO content to saturation at 20°C. Agitate or aerate with clean, filtered compressed air.

Dilution of sample: Dilutions that result in a residual DO of at least 1mg/L and DO uptake of at least 2mg/L produce reliable results. Make several dilutions of the pre-treated sample so as to obtain about 50% depletion of DO or DO uptake of 2mg/L. Prepare dilutions 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 mixed sample and dilute to the appropriate volume by siphoning dilution water. Mix well with plunger type mixing rod to avoid entraining air.

General guidelines for dilution range are as follows:

0.1% to 1% : Strong trade waste

1% to 5% : Raw or settled sewage

5% to 25% : Treated effluent

25% to 100% : River water

Sample processing:

a. Siphon the diluted or undiluted sample in three labeled bottles and stopper immediately.

b. Keep 1 bottle for determination of the initial DO and incubate 2 bottles at 20°C for 3days. See that the bottles have a water seal.

c. Prepare a blank in triplicate by siphoning plain dilution water (without seed) to measure the O_2 consumption in dilution water.

d. Also prepare a seed blank in triplicate to measures BOD of seed for correction of actual BOD.

e. Determine DO in a BOD test can in the blank on initial day and end of incubation period by Winkler method as described for DO measurement.

f. DO estimation in a BOD test can also be done by membrane electrodes. A DO probe with a stirrer is used to determine initial and final DO after incubation in BOD samples. The semi-permeable membrane provided in the DO probe acts as a diffusion barrier against impurities between sensing element and sample.

G. Iron

Presence of iron causes nuisance in public water supplied. It occurs both in soluble and insoluble forms. When the iron is present in considerable amounts in water it imparts colour and also develops turbidity when exposed to air due to its conversion to ferric state. Hence, the water becomes

unacceptable for drinking purposes from an aesthetic point of view. Further, it interferes with laundering operation, imparts objectionable stains, causes difficulties in the distribution systems and imparts taste even at low concentrations (0.3 mg/l).

Principle

The ferric form of iron is reduced to ferrous form by boiling with HC1 and hydroxylamine hydrochloride. Later phenanthroline is added at pH between 3.2 and 3.3 to form soluble chelated complex of orange red colour with iron. The colour obeys Beer's Law and the intensity of colour is independent of pH between 3 and 9.

Interference

Stron oxidizing agents such as CN, NO_2, polyphosphates, Cr, Zn in Conc. 10 times the Fe conc; Co and Cu if 5 mg/l, Ni if 2 mg/l colour and organic matter constitutes sources of interference in the development of colours. Boiling with HCl and addition of hydroxylamine remove interferences due to CN, NO_2, PO_4 and other oxidizaing reagents. The metal ions get complexed with phenanthroline.

Apparatus

1. Colorimeter with an operating range of 400-700 mu.
2. Nessler's tubes.

Reagents

1. HCl cone.
2. Hydroloxylamine soloution: Dissolve 10g NH_2 OH HCl and dilute to 100 ml.
3. Ammonium acetate buffer: Dissolve 250 g $NH_4C_2H_3O_2$ in 50 ml distilled water. Add 700 ml conc. Glacial acetic acid. Final volume will be slightly more than 1000 ml.
4. Phenanthrolone solution: Dissolve 100 mg/l: 10 phenanthroline monobydrate in 100 ml distilled water warm slightly or add 2 drops cone. HCl if necessary. 1 ml of this colution can chelate 100 mg iron.
5. Stock iron solution: Add 20 ml conc. H_2SO_4 to 50 ml distilled water and dissolve 1.404 g Fe $(NH_4)_2$ $(SO_4)_2$ $6H_2O$. Add drop-wise 1 N KM nO_4 till faint pink colour persists. Dilute to 1000 ml. 1 ml = 200 mg Fe.
6. Standard iron solution: Dilute 50 ml stock Fe solution to 1000 ml. Prepare this solution freshly. 1 ml = 10 mg Fe.

Procedure

1. Take suitable aliquot (about 50 ml having 2 mg/l Fe) of well mixed sample in 125 ml conical flask.
2. Add 2 ml conc. HCl followed by 1 ml hydroxyl amine solution.

3. Add2-3 glass beads and boil for 20-25 min. to ensure dissolution of Fe.
4. Cool to room temp. and transfer to Nessler's tubes.
5. Add 10 ml amm. acetate buffer and 2 ml phenanthroline solution.
6. Dilute to 100 ml and mix well.
7. Prepare blank by substituting the sample by water.
8. For soluble iron determination, take known vol. of filtered sample, acidify by adding 2 ml. Conc. HCl per 100 ml of sample and treat from step 5 onwards for colour development.
9. Prepare calibration curve taking standard iron solution in the same way in the range 1000-4000 mg/l with 1 cm light path.
10. Measure the developed colour after 10 mn. at 510 mu.
11. Calculate the concon. of total or soluble Fe present in the sample from caliberation curve and express as mg/l.

H. Fluoride

Fluoride ions have dual significance in water supplied. Excess concentration of F causes dental fluorosis (disfigurement of the teeth). At the same time, a concentration less than 0.8 mg/l results in `dental caries'. Hence, it is essential to maintain the F conc. Between 0.8 to 1.0 mg/l in drinking water.

Principle

Under acid condition fluorides (HF) react with zirconium SPADNS solution and the lake (colour of SPADNS reagent) gets bleached due to formation of ZRF_6. Since bleaching is a function of fluoride ions, it is directly proportional to the conc. of F. It obeys Beer's Law in a reverse manner.

Interference

Alkalinity 5000 mg/l, aluminium 0.1 mg/l, chloride 7000 mg/l. Fe 10 mg/l, PO_4 16 mg/l, SO4 200 mg/l and hexametaphosphate 1.0 mg/l interfere in the bleaching action. Residual Cl2 interferes in bleaching action. In presence of interfering radicals distillation of sample is recommended.

Apparatus

1. Colorimeter for use at 570 M/U.
2. Nessler's tubes cap. 100 ml.

Reagents

1. SPADNS solution: Dissolve 958 mg SPADNS and dilute to 500 ml.
2. *Zirconyl acid ragent*: Disslove 133 mg $ZrOCL_2$. $8H_2O$ in 25 ml water. Add 350 ml conc. HCl and dilute to 500 ml.
3. Mix equal volume of 1 and 2 to produce a single reagent. Protect from direct light.
4. *Reference solution*: Add 10 ml SPADNS solution to 100 ml distilled water. Dilute 7 ml conc. HCl to 10 ml and add to diluted SPANDS solution.
5. *Sodium arsenite solution*: Dissolve 15.0 g $NaASo_2$ and dilute to 1000 ml.

6. *Stock* F *solution:* Dissolve 221.0 mg anhydrous NaF and dilute to 1000 ml. 1 ml = 100 mg F.
7. *Standard* F: Dilute stock solution 10 times to obtain 1 ml = 10 mgF.

Procedure

1. Prepare standard curve in the range 0.0 to 1.40 mg/l by diluting appropriate volume of standard F solution to 50 ml in Nessler's tubes.
2. Add 10.0 ml mixed reagent prepared as in 3 above to all the samples, mix well and read optical density to bleached colour at 570 mu using reference solution for setting zero absorbance.
3. Plot cone. vs % transmission.
4. If sample contains residual chlorine remove it by adding $NaAsO_2$ solution 1 drop (o.05 ml = o.1 mg Cl) $NaAsO_2$ conc. should not exceed 1300 mg/l to avoid error due to $NaAsO_2$.
5. Take suitable aliquots of sample and dilute to 50 ml in Nessler's tubes.
6. Add mix reagent 10 ml; mix well and read % transmission.
7. Calculate the mg F present in the sample using standard curve.

Box.1. Definitions

Normality (N)

Normality is another way of expressing the concentration of a solution. It is based on an alternate chemical unit of mass called the equivalent weight. The normality of a solution is the concentration expressed as the number of equivalent weights (equivalents) of solute per liter of solution. A 1 normal (1 N) solution contains 1 equivalent weight of solute per liter of solution. Every substance may be assigned an equivalent weight. The equivalent weight may be equal to the formula weight (molecular weight, mole weight) of the substance or equal to an integral fraction of the formula weight (i.e., molecular weight divided by 2, 3, 4, and so on).

Molarity (M)

A mole is defined as "The amount of substance containing the same number of particles as the number of atoms present in 0.012 kg (or 12 gms) of carbon-12".Or Mole is the quantity of substance that contains 6.023×10^{23} particles (Avogadro's number)

Components of Solution

In the study of solution, it is customary to designate the components in solution. The components are Solvent (The component present in larger proportion is known as solvent), Solute (The component present in smaller proportion is known as solute), Solution = Solvent + Solute

Amount of solute require to prepare Normal solutions = Normality of solution required X Equivalent weight of solute x Volume of solution required / 1000

For Diluting any solutions/ reagents formula used is V1N1 = V2 N2

Where V1= Volume of secondary solution
N1= Normality of secondary solution
V2= Volume of primary solution to be taken
N2= Normality of secondary solution

Water quality Bureau of Indian standards (BIS)

PARAMETER	Highest Desirable level	Maximum permissible level
A. Physico-Chemical		
Electrical conductivity at 25^0C µs/cm	750	3500
Total solids (mg/l)	500	2000
Colour (Hazen Units)	5	30
Taste	Unobjectionable	-
Odour	Unobjectionable	-
Turbidity (NTU)	2	8
Chloride (Cl^-) (mg/l)	200	1200
Fluoride (F^-) (mg/l)	-	1.5
Iron (Fe) (mg/l)	0.3	1
Manganese (Mn) (mg/l)	0.05	0.5
Copper (Cu) (mg. I)	0.05	1.5
Zinc (Zn) (mg/l)	5	15
Calcium (Ca) (mg/l)	100	240
Magnesium (Mg) (mg/l)	30	150
Total Phosphates (PO_4^{3-}) (mg/l)	-	2.0
Sulphate (SO_4^{2-}) (mg/l)	200	400
Total Alkalinity (as $CaC0_3$) (mg/l)	200	400
Total Hardness (as $CaC0_3$) (mg/l)	250	600
Free Ammonia (as NH_3) (mg/l)	-	0.06
Nitrate (NO_3^-) (mg/l)	-	45
Nitrite (NO_2^-) (mg/l)	-	0.01
pH	7.0 – 8.5	6.5 – 9.0
Arsenic (As) (mg/l)	-	0.05
Cadmium (Cd) (mg/l)	-	0.005
Chromium (Cr) (mg/l)	-	0.05
Cyanide (CN^-) (mg/l)	-	0.05
Lead (Pb) (mg/l)	-	0.05
Mercury (Hg) (mg/l)	-	0.001
Selenium (Se) (mg/l)	-	0.01
Free Residual Chlorine (as Chlorine) (mg/l)	-	0.2
Polynuclear aromatic hydrocarbons (mg/l)	-	0.0002
Phenolic compounds (as phenolic OH) (mg/l)	0.001	0.002
Grease & Oil (mg/l)	-	1.0
COD (Chemical Oxygen Demand) (mg/l)	-	10
Radioactive materials		
Gross alpha radioactivity (pC/I)	-	3
Gross beta radioactivity (pC/I)	-	30
B. Bacteriological		
Total Coliforms / 100 ml	Absent in (i) 95% of the samples in a year and (ii) in any two consecutive samples	10
E.Coli/100ml	Absent	Absent

MICRO BIOLOGICAL ANALYSIS OF DRINKING WATER

The most common and widespread health risk associated with drinking water is contamination directly or indirectly, by human or animal excreta, particularly faces.This contamination may result in the growth of pathogenic microorganisms such as bacteria, viruses, and protozoa, which may cause diseases/infection by drinking the water or by using it for food preparation.

These bacteria (coli forms) are found in the intestines of warm blooded animals and therefore are present in sewage, in soils, surface waters and vegetation. The coli form group has been used as an "indicator organism".

Coli forms are a group of bacteria which is gram-negative, rod shaped bacteria which ferment lactose with gas formation within 48 hours at 37°C. These are found in soil, vegetation, animal and human waste contaminated water. Fecal coli forms are found in animal and human waste contaminated water. They have the ability to ferment at 44.5°C ferment lactose with gas formation within 48 hours. *E. coli* (*Escherichia coli*) is one of the fecal coli form bacteria.

By measuring the number of total coli form present in a sample a judgment can be made as to the water's usability for a given purpose.

BACTERIAL DISEASES

Disease	Pathogen	Symptoms	Transmission
Campylo bacteriosis	*Campylobacter jejuni*	Diarrhea, stomach pain, and stomach cramping	Chicken, unpasteurized milk, water
Cholera	*Vibrio cholera*	Watery diarrhea, vomiting, and leg cramps	Contaminated drinking water, rivers and coastal waters
E. Coli	*Escherichia coli*	Diarrhea (may be bloody), abdominal pain, nausea, vomiting, fever, HUS	Undercooked ground beef, imported cheeses, unpasteurized milk or juice, cider, alfalfa sprouts
Hepatitis A	Hepatitis A virus	Fever, fatigue, stomach pain, nausea, dark urine, jaundice	Ready-to-eat foods, fruit and juice, milk products, shellfish, salads, vegetables, sandwiches, water
Legionellosis	*Legionella pneumophila*	Fever, chills, pneumonia, anorexia, muscle aches, diarrhea and vomiting	Contaminated water
Salmonellosis	*Salmonella typhi*	Abdominal pain, headache, fever, nausea, diarrhea, chills, cramps	Poultry, eggs, meat, meat products, milk, smoked fish, protein foods, juice
Vibrio Infection	*Vibrio parahaemolyticus*, *Vibrio vulnificus*	Nausea, vomiting, headache (a quarter of patients experience dysentery-like symptoms)	Raw shellfish, oysters

MOST PROBABLE NUMBER (MPN) METHOD

The MPN method attempts, by serial dilution, to introduce the bacteria into a fermentation tube containing lactose broth for the bacteria to thrive on. By observing gas production or the lack of gas production, it is possible to determine the probable number of bacteria originally present in the sample.

SAMPLE COLLECTION PROCEDURE

1. Sterilize the bottles at 121^{0}C under15 lbs pressure for 20 min (autoclave)/ pressure cooker.
2. Keep the sample bottle unopened after sterilization until the sample is to be collected.
3. Carefully unscrew the cap of the sterile bottle and immediately collect the samples and a small air space are left to make shaking before analysis.
4. Collected sample delivered to laboratory within 6 hours and inoculated immediately.

Medias required: (all are readymade medias)

1. Lactose broth
2. BGLB broth
3. Peptone broth
4. EMB agar

METHOD FOR TESTING COLIFORMS

This test consists of two to three steps:

1. Presumptive test
2. Confirming test
3. Completed test

Presumption test:

1. Arrange three double strength and six single strength 10 ml tubes containing Lactose broth and Durham tubes.
2. Inoculate 10ml of the sample in double strength tubes, 1.0ml of the sample in three single strength tubes and 0.1ml of the sample in another three single strength tubes.
3. The tubes are kept in the incubator at 37^{0}C for 24 to 48 hours for the detection of total coli form bacteria and incubate at 44^{0}C for fecal coli form bacteria.
4. The presence of bacteria will show gas production or color change/acid production of the broth.
5. The number of bacteria was counted based on the number of positive tubes in each set and compared with standard chart.

Conformation test:

1. Inoculate from the positive tube in presumption test to Brilliant green lactose bile broth with Durham tubes.
2. Incubate the tubes the incubator at 37^{0}C for 24 to 48 hours.
3. Presence of gas production confirm the presence of bacteria

Conformational test for *E. coli*:

1. Inoculate from the positive tube from presumption test into EC broth which contains Durham tubes.
2. Incubate the tubes in the incubator at 37^{0}C for 24 to 48 hours.
3. Presence of gas production confirm the presence of *E. coli*

Completed test for *E. coli*

1. Inoculate a loop of culture from the positive tube of conformation test on EMB (Eosin methylene blue) agar.
2. Incubate the plates in the incubator at 37^0C for 24 to 48 hours.
3. Green-golden metallic sheen colonies provide positive confirmation of *E. coli*

MPN DETERMINATION FROM MULTIPLE TUBE TEST

NUMBER OF TUBES GIVING POSITIVE REACTION OUT OF			MPN INDEX	95 PERCENT CONFIDENCE LIMITS	
3 of 10 ml each	3 of 1 ml each	3 of 0.1 ml each	per 100 ml	LOWER	UPPER
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1300
3	3	1	460	71	2400
3	3	2	1100	150	4800

FROM: STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, TWELFTH EDITION. (NEW YORK: THE AMERICAN PUBLIC HEALTH ASSOCIATION, INC., p. 608)