DELHI JAL BOARD

(GOVT. OF N.C.T. OF DELHI)

KAMRUDDIN PLANT 40 MGD (W.T.P)

Under Supervision of
Mr Sahdev Kr. Rathl (ACWA)
Mr Shailesh Pandey (ACWA)

INTERNSHIP REPORT

DSEU
Crafting Excellence

GURU NANAK DEV DSEU ROHINI CAMPUS

SUMITTED BY : Himanshu Gupta

ROLL NO : 10421036

BRANCH : CHEMICAL ENGINEERING

TRAINING PERIOD : 1MONTH

Acknowledgement

I would like to express my profound gratitude to **Sh. Sahdev Kumar Rathi (ACWA) & Sh. Shailesh Pandey (ACWA),** of Zonal Laboratory Nangloi, Delhi Jal Board, for their contributions to the completion of Internship Training.

I would like to express special thanks to my mentors **Mr.Shashi Bhushan (Bacteriologist, NWS), Mr.Sagar (Assistant Chemist, DJB), and Mr.Prateek (Bacteriologist, NWS)** for their time and efforts they provided throughout the period. Your useful advice and suggestions were really helpful to me during the training's completion. In this aspect, I am eternally grateful to you.

Signature

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INTRODUCTION

It is a relative term used to convey the idea of the potential usability of ground surface water for a particular use.

- The quality of water is determined by its physical characteristics (temperature, colour, turbidity, taste, odour etc).

- Water is an important gift of nature for all living things. As it is used in all types of metabolic activities and house hold works. It may be defined as “Clear, transparent, Odourless and tasteless liquid chemically it is made up of two atoms of Hydrogen and on atom of Oxygen.

- In day to day life, required portable water means should be tasteful ,aesthetically pure and wholesome . But due to presence of some chemicals , biological and microbes it’s becomes unfit for drinking and for other uses

- In our Earth only 0.1% water is available for our day to day use in the form of river, ponds, lakes and underground water . Rest 99.9% water is either in sea oceans or in the form of Glacier.

- Out of this 0.1% water, most of the sources are not fit for drinking due to different types of contamination pollution.

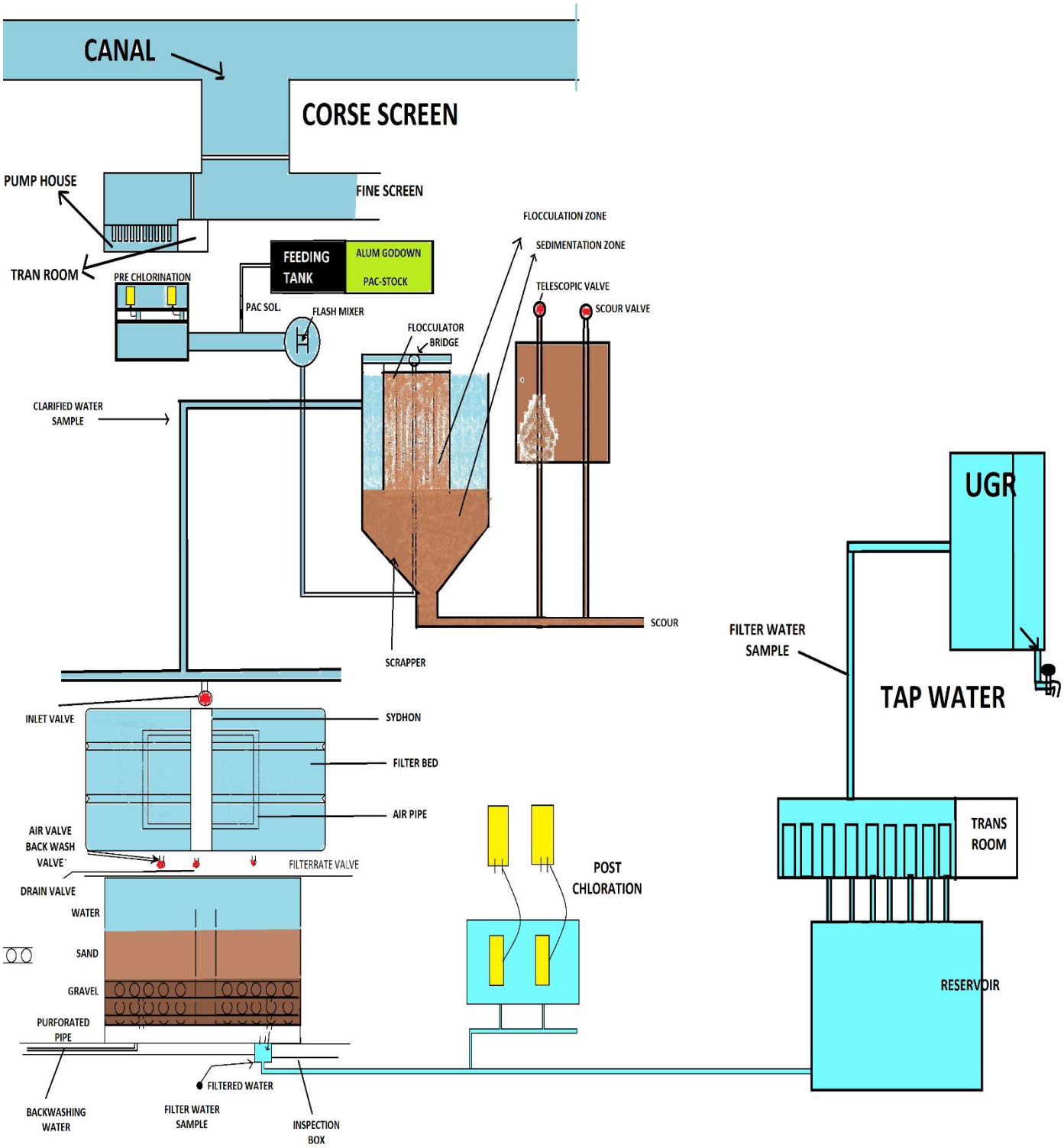
- Water pollution may be defined as “Any physical Chemical or biological changes in water (Either by natural or man-made sources). This water causes undesirable effects on living organisms because due to toxicity of water and reduction of dissolved Oxygen.

- Generally, two types of sources cause water pollution
    - Point source pollution
    - Non-point source pollution

Conventional Water Treatment & Quality control

- Intake of Raw Water from River/Canal by gravity.
- Screening Floating material is removed as it creates hindrance in treatment & pollution by its by products.
- Pumping of Raw Water to the plantRaw water is pumped to the common sump so that water can flow by gravity in the plant.
- Pre Chlorination disinfection---Pre chlorination is don to meat the demand of Raw water, killing of microbes, algae, oxidation of Ammonia, Iron, Manganese, colour bleaching & odour removal.
- Addition of coagulant ALUM/PAC-coagulant added as per dose to remove suspended solids like mud, silt etc by coagulation (precipitation), flocculation & sedimentation.
- Addition of lime & copper Sulphate-if required-lime is added if p H is very low (<7) in Raw water & copper sulphate is added if algael load is high.
- Flash mixer coagulation) --In this unit mechanical & chemical action is going on (homogeneous mixing & precipitation)
- Flocculation - The coagulated water goes in flocculation zone & by mechanical action flocculation takes place & make settlable flocs.
- Clarification (sedimentation-separation of solid & liquid in sedimentation zone. The supernatant water comes out from the clarifier.
- Scouring of sediment of clarifier-The sedimented sludge from the bottom removed by scouring by hydraulic pressure.
- Filteration (Rapid sand gravity filter-The clarified water goes in filter bed & filtered through 44 inch filter media. The mud particles retain on the bed.
- Back washing of filter-After 30 to 40 hours running the rate of filteration reduced. Hence regeneration of bed is required. For this 3 minute air to agitate) & back wash water from the bottom comes out of the filter draining mud in the gutter.
- Post chlorination-disinfection Chlorine is added at the rate of 1.5 mg/l to maintain Res/ch 0.2 mg/l at consumer end.
- Storage UGR-The final water is filled in reservoir.
- Clear water pump house --- Potable drinking water now is pumped to the city as per requirement.
- Surveillance of water quality from command area The water samples collected daily from area for bacteriological & chemical analysis in laboratory.
- Leak detection & Rectification / disinfection of rising, mains, service lines UGR, OHT.
- Total analysis, dosing of chemicals, operation of treatment units and process control, quality control & maintain potable quality up to consumer end is ensured.

WATER TREATMENT PROCEDURE



SPECIFICATION FOR SOME OF THE IMPORTANT

S.NO	CHARACTERISTIC	UNIT	REQUIREMENT	PERMISSIBLE LIMIT
1	**Colour**	Hazen	5	15
2	**Odour**	--	Agreeable	Agreeable
3	**Turbidity**	N.T.U	1.0	5.0
4	**Taste**	--	Agreeable	Agreeable
5	**Ph Value**	--	6.5-8.5	No Relaxation
6	**Total Dissolved Solids**	Mg/L	500	2000
7	**Total Alkalinity**	Mg/L	200	600
8	**Total Hardness**	Mg/L	200	600
9	**Chloride**	Mg/L	250	1000
10	**Fluoride**	Mg/L	1.0	1.5
11	**Sulphate**	Mg/L	200	400
12	**Ammonia**	Mg/L	0.5	No Relaxation
13	**Nitrate**	Mg/L	45	No Relaxation
14	**Calcium As Ca**	Mg/L	75	200
15	**Magnesium As Mg**	Mg/L	30	100
16	**Aluminium**	Mg/L	0.03	0.2
17	**Iron**	Mg/L	1.0	No Relaxation
18	**Boron**	Mg/L	0.5	1.0
19	**Manganese As Mn**	Mg/L	0.1	0.3
20	**Copper As Cu**	Mg/L	0.05	1.5
21	**Zinc**	Mg/L	5	15
22	**Phenolic Compounds**	Mg/L	0.001	0.002
23	**Total Arsenic**	Mg/L	0.01	0.05
24	**Cadmium**	Mg/L	0.003	No Relaxation
25	**Total Chromium**	Mg/L	0.05	No Relaxation
26	**Cynide**	Mg/L	0.05	No Relaxation
27	**Lead**	Mg/L	0.01	No Relaxation
28	**Mercury**	Mg/L	0.001	No Relaxation
29	**Res. Chlorine**	Mg/L	0.2	1.0
30	**Coliform Count**	No./100ml	NIL	NIL

PARAMETERS

SAMPLING

Water sample testing analyses water samples to determine their physical and biochemical properties.
Water samples are taken from different locations within the water distribution system**,** including water treatment plants, storage tanks, tap water, or a natural spring. The water is then tested for the presence of specific contaminants**.**

Water sample testing is integral to water quality management and helps ensure that drinking water is safe for use or consumption.

Water testing can be done for a variety of reasons. For the most part, the reasons are:

- assessing water quality
- investigating water pollution

The Purpose of Water Sample Testing

Water nowadays can come from various sources, including ditches, streams, or treatment plants. Sometimes, this water can be contaminated. It may come in contact with bacteria, dissolved metals, viruses, household and plant sludge, or other elements that may prove harmful once ingested by humans.

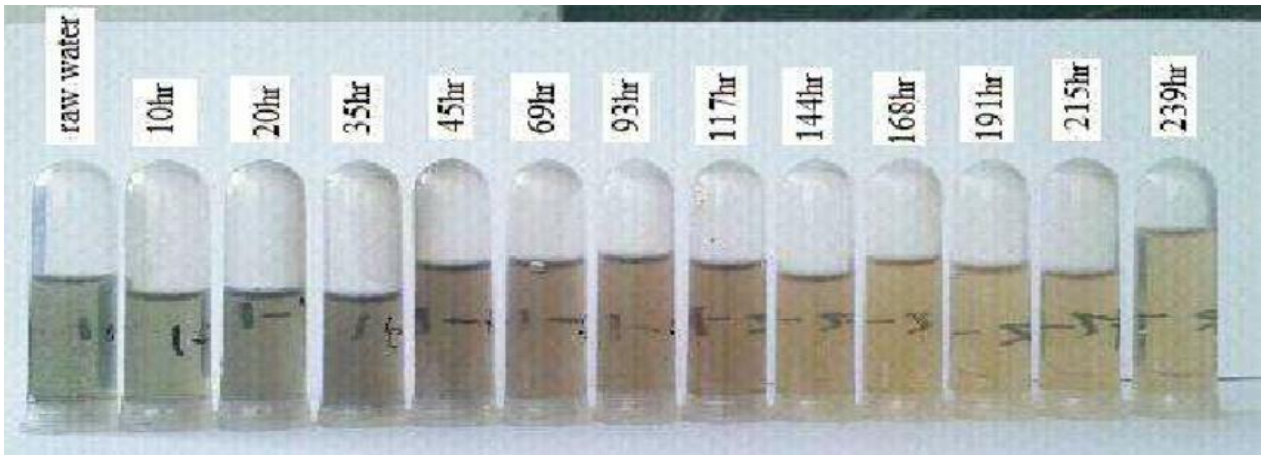
Types of Samples
There are two types of water sampling strategies regarding the time frame when the samples are collected:

- discrete samples
- composite samples.[1]

Discrete sample, also known as grab sample, is a single sample collected in an individual container. The sample is representative of the chemistry only at the time and place at which the sample was taken. The time period is generally defined to be less than 15 min. Thus, discrete samples are appropriate when the sample composition is not time dependent.

Composite sample consists of a series of smaller samples collected at a predetermined time or after predetermined flow and mixed in the same container.

COLOUR



Color in natural water is caused by metallic substances like iron and manganese, humus peat tannins, algae, weeds, and protozoa. Color means true Color which is due to the substances present as fine collides. Apparent Color is due to both suspended and dissolved matters.

METHOD:- The platinum cobalt method of measuring Color is the standard method, the unit of Color being that produced by. 1 mg platinum/ liter in the form of chloroplatinate ion. It is applicable to measure the Color of potable water and of water in which Color is due to naturally occurring materials.

REAGENTS:- Dissolve 1.246 gm Potassium chloroplatinate, K,PtCl (equivalent to 500 mg metallic Pt). and 1.00 g. crystallized CoClO (equivalent to about 250 mg metallic Co) in distilled 22 water with 100 ml conc. HCl and dilute to 1000 ml distilled water. This stock standard has a Color of 500 units. Prepare standards having colors of 5, 10, 15, and 20, Color standard with distilled water to 50 ml in Nessler tubes. 70 by diluting the stock.

PROCEDURE:- Observe sample Color 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 Color.

ODOUR

Odour is important parameter but can not be expressed in absolute unit: It varies person to person and intensity of individual hence opinion of several observer may be-recorded.Take 500 ml sample in clear odourless wide mouth glass bottle of one liter shake and immediately obse the odour The water sample may also be warmed up to 60 degree centigrade for observation of odour. Odour may be as rotten egg, burnt sugar, soapy, fishy, septic, alcoholic, aromatic, chlorinous, sew Sulphury, algael, etc may be reported as agreeable or disagreeable.

PH VALUE

Testing the pH value of a substance, such as water or a solution, is a straightforward process that helps determine its acidity or alkalinity. pH is measured on a scale from 0 to 14, where 0 represents highly acidic, 7 is neutral, and 14 is highly alkaline.

Apparatus:-

- pH Meter (manufactured manufacturing company)by and reputed international calibrated by NABL accredited lab).
- Beakers
- Magnetic stirrer

Reagents:

1. Acetic buffer, pH 4.62:
2. Phosphate Buffer, pH 7.0
3. Borate Buffer, pH 9.0:

Procedure:

- After calibration, rinse the electrode with distilled water and wipe gently by blotting with a soft tissue.
- Take the sample in a beaker. Bring the temperature of the sample to room temperature.
- Place the beaker on a magnetic stirrer. Bring the ample to homogeneity by stirring.
- Record the reading, which will give the pH value of the sample.

CONDUCTIVITY

Apparatus:-

1. Conductivity meter with conductivity probe
2. Beakers
3. Wash bottle

Reagents:-

1. Potassium Chloride Standard Solution, KCl, 0.01M

Procedure:-

1. Follow manufacturer's instructions for calibration or use of the instrument and for storage of the probe.

2. Before use, rinse the probe with DDW. Dry probe by gently blotting with a soft tissue.

3. Place the probe in standard potassium chloride, KCl, 0.01 M solution and make sure the slot on the end of the probe is totally immersed. Agitate the solution with probe for 5-10 seconds to remove bubbles that may be trapped in the slot.

Conductivity of the above solution=1413 umhos/em

1. Now place the probe into the sample and make sure the slot on the end of the probe totally immersed. Agitate the sample with the probe for 5-10 seconds to remove bubbles that may be trapped in the slot.

TOTAL DISSOLVED SOLUTION (TDS)

Testing TDS (Total Dissolved Solids) using conductivity is a widely used method to estimate the concentration of dissolved substances in water. The test is based on the principle that the electrical conductivity of water increases with the amount of dissolved ions and substances. Here's a general outline of how to perform a TDS test using a conductivity meter

Materials and Equipment:-

- Sample of water to be tested
- Clean sample bottle or container
- Conductivity meter (TDS meter)
- Calibration solution for the conductivity meter
- Stirring rod or paddle (optional)

Procedure:-

- Calibration (if applicable): Before testing, calibrate your conductivity meter using a calibration solution with a known conductivity value. The calibration process ensures the accuracy of the measurements. Follow the manufacturer's instructions for calibration.
- Sample Collection: Collect a representative sample of the water to be tested in a clean sample bottle or container. Ensure that the container is thoroughly rinsed with the water to be tested to avoid any contamination.

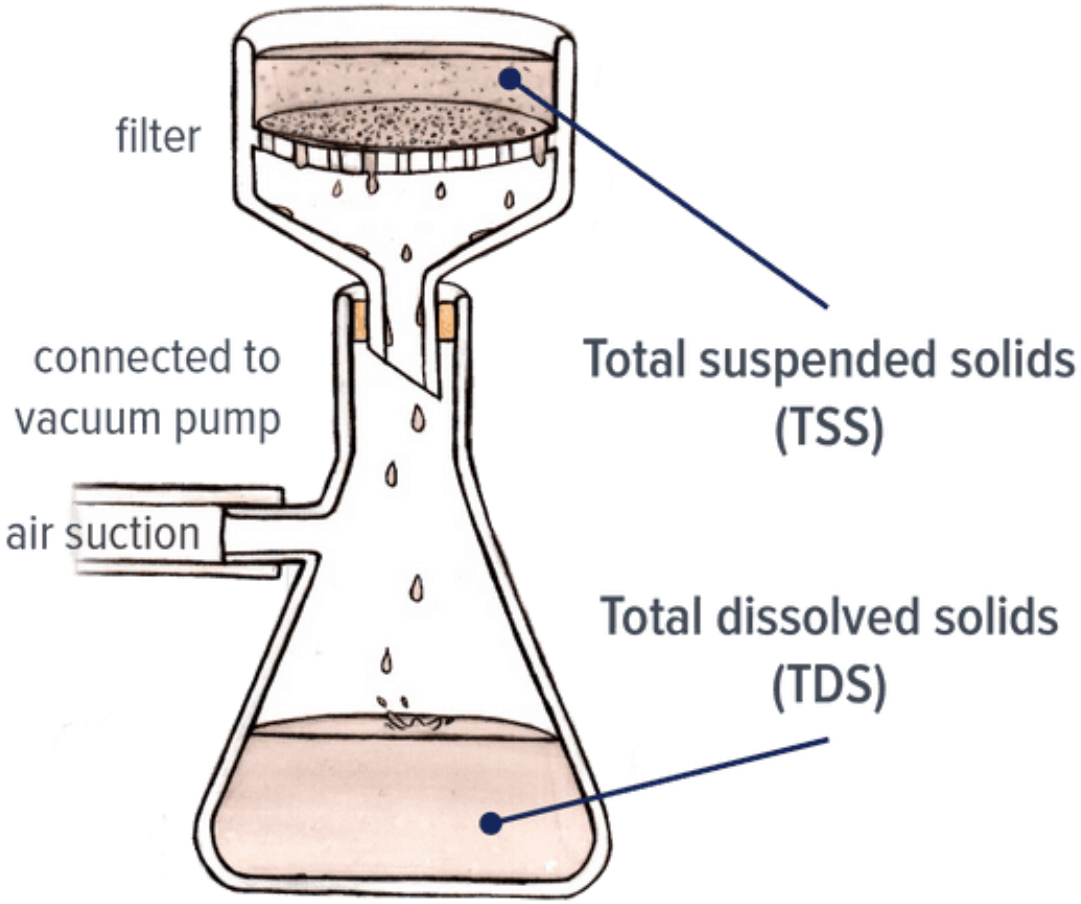
Many conductivity meters have the capability to convert the conductivity reading directly to TDS using a built-in factor or algorithm. However, if your meter does not provide this feature, you can use a TDS conversion factor specific to your water source or follow a general conversion factor provided by the manufacturer.

It's important to note that while measuring TDS through electrical conductivity provides a quick estimate of the dissolved substances in the water, it does not identify the specific ions or substances present. Further testing or analysis may be required to identify the individual components of the TDS.

Always follow the manufacturer's instructions for operating the conductivity meter and calibrating it properly to obtain accurate and reliable TDS measurements. Regular TDS testing is essential for assessing water quality.

CALCULATION:-

$$T.D.S = CONDUCTIVITY \times 0.64$$



TOTAL ALKALINITY

The total alkalinity test is a common water quality analysis performed to determine the concentration of alkaline substances in water

Apparatus:-

1. Volumetric flasks- from 100-ml to IL capacity
2. Volumetric pipettes- graduated
3. Digital burette calibrated.
4. Erlenmeyer flasks- 250 ml capacity

Reagents:-

1. Stock Sodium Carbonate Solution; Na2CO3,, 0.1N
2. Standard Sodium Carbonate Solution; Na2CO3,, 0.02 N
3. Standard Sulphuric Acid; H_2SO4, 0.1N
4. Standard Sulphuric Acid; H_2SO4, 0.02N
5. Methyl Orange Indicator.
6. Phenolphthalein Indicator

Procedure:-

- Take 50 ml of unfiltered sample into a 250-ml; conical flask.
- Record initial pH and temperature.
- if initial pH is more than 8.3, titrate with 0.02N H_2SO4, using phenolphthalein indicator, till the pink colour disappears. This is phenolphthalein end point. However, if the pH of sample is less than 8.3 and no change in colour is observed after the addition of phenolphthalein indicator, proceed for next steps.
- Add the 2-3 drops of mixed indicator and titrate with 0.02N H_2SO4, till pinkish colour is obtained, record the reading, this is total alkalinity (T).

It's important to note that there are different methods for measuring total alkalinity, such as the Gran method, phenolphthalein method, and pH endpoint method, among others. Each method may require specific reagents and procedures, so it's essential to follow the appropriate standard method for accurate results.

TOTAL HARDNESS

Testing for total hardness in water is a common water quality analysis performed to determine the concentration of calcium and magnesium ions, which are the primary contributors to water hardness.

Apparatus:-

1. Volumetric Flask
2. Digital Burette
3. Graduated Pipettes

Reagents:-

- Buffer Solution
- Standard Calcium Solution
- Eriochrome Black T Indicator Solution
- Inhibitor
- Standard EDTA titrant, 0.01 M

Procedure:-

Standardization of 0.01 M E.D.T.A. Solution:-

1. Take 10.0 mL 0.01M $CaCO_3$, in a conical flask. Add 02 mL of buffer solution. Add pinch of EBT. Wine Red Color Appear.
2. Titrate against 0.01M E.D.T.A., at the end point sky blue color appears.

Total Hardness:

1. Pipette out 50.0 mL sample or suitable dilution to 50 mL in a conical flask. Add 01 mL hydroxylamine hydrochloride ($HONH_2.HCl$). Add 01 to 02 mL buffer solution so as to achieve pH 10.0 to 10.1
2. Add Eriochrome Black T pinch as indicator. Titrate with standard EDTA solution stirring rapidly in the beginning and slowly towards the end till the end point is reached when all the traces of red and purple color disappear and disappear and solution is clear sky blue in color.

It's important to note that this is just one of the methods used for total hardness testing. Other methods may use different reagents and indicators. Always follow the specific instructions provided with the test kit or method you are using for accurate results.

CHLORIDE

Testing for chloride in water is a standard water quality analysis performed to determine the concentration of chloride ions (Cl^-) in a water sample. Chloride testing is important as elevated chloride levels can indicate contamination from various sources, such as road salt, sewage, or industrial discharges.

Apparatus:-

1. Volumetric Flask
2. Digital Burette
3. Graduated Pipettes

Reagents:-

1. Potassium Chromate indicator solution
2. Standard Silver Nitrate: N/35.5
3. Standard Sodium Chloride; N/35.5

Procedure:

1. Sample Preparation: Use a 5-mL sample or a suitable portion diluted to 50 ml.
2. Titration: Add 1.0 mL. K2CrO4, indicator solution. 3. Titrate with standard AgNO3, titrant to a pinkish yellow end consistent in end-point recognition.
   Standardize AgNO3 titrant and establish reagent blank value by the titration method outlined above. A blank of 0.2 to 0.3 mL is usual.

It's important to note that this is just one of the methods used for chloride testing. Other methods may use different reagents and indicators. Always follow the specific instructions provided with the test kit or method you are using for accurate results.

TURBIDITY

Testing the turbidity of water is a standard procedure used to measure the cloudiness or haziness caused by suspended particles in the water. High turbidity levels can impact water quality and can indicate the presence of contaminants. The turbidity test can be performed using a turbidimeter or a simple turbidity tube.

Nephelo metric Turbidity Meter is used to measure the turbidity of the water sample .

Procedure:

- For best results, always cover vial with vial cover whenever measuring a sample.
- Allow the sample to come to room temperature.
- Mix the sample thoroughly to disperse the solids.
- Wait until all visible air bubbles disappear (a few minutes at most).
- Select the measurement mode.
- Pour the sample into a clean, dry turbidity vial.
- If the sample has settled, mix it gently to re-suspend the before pouring it into the sample vial.
- Cap the vial securely.
- Wipe the vial free of liquid and fingerprints with a soft lint-free wipe or cloth.
- Place the vial into the sample chamber and cover it with the vial cover.
- Press the key.
- The meter will display the result. Record the value or log the data Proceed with the next sample.

High turbidity levels can indicate water quality issues, so it's essential to monitor and control turbidity, especially in drinking water sources and other critical applications. Regular turbidity testing is vital for ensuring safe and clean water.

AMONNIA

Testing for ammonia in water is a crucial water quality analysis as ammonia can be harmful to aquatic life and indicate the presence of organic or chemical pollutants.

Apparatus:-

- Spectrophotometer for use at 415 nm, providing a light path of at least 1 cm.
- Nessler Tube-100 mL Capacity
- Volumetric Flask

Reagents:

- Rochelle salt:
- Nessler's Reagent

Stock Ammonia Solution: 1 mL = 1.0 mg N= 1.22 mg NH, Working Ammonia Solution: 1 mL=0.01 mg N

Sample Testing:-

1) 50 ml D.W. & 50 mL Sample in different Nessler tubes.
2) Add 1 mL Rochelle salt to each.
3) Add 1.0 ml Nessler Reagent to each.
4) Mix Well
5) Reaction time - 10 min
6) Read the Zero
7) Read the sample

NITROGEN (NITRITES)

Apparatus:-

- Colorimeter or Spectrophotometer that can be operated at 520 am.
- Nessler tubes- 100 ml. Capacity , c. Volumetric flask.

Reagents:-

- Sulphanilic Acid Solution
- Alpha-Naphthylamine Hydrochloride Solution
- Sodium Acetate Buffer Solution:
- Stock Nitrite Solution: 1 ml 0.05 mg of nitrite as N e. Standard Nitrite Working Solution: 1 ml = 0.0005 mg of nitrite as N

.Procedure:-

- If sample contain suspended solid, then coagulate the sample with ZnSO4, and NaOH as in ammonia and clear supernatant sample.
- Take 50 ml or suitable dilution of 50 ml sample in Nessler tube and add 1.0 ml Sulphanilic acid reagent and mix.
- At this point pH should be 14 then add 1 ml a-Naphthylamine Hydrochloride Solution.
- Add 1 ml sodium acetate buffer and mix well.
- At this point pH should be 2.0 to 2.5 after 10 min. Compare with a series of standard nitrite solution or see transmission at 520 am because it follows beer's law

CHLORINE DEMAND

Total chlorine demand testing is an essential process in water treatment to determine the amount of chlorine required to achieve the desired disinfection level in water. It helps water treatment facilities optimize chlorine dosing and maintain an adequate disinfectant residual throughout the distribution system.

Principle:-

Chlorine demand is that amount of chlorine which has to be added to water to reach the break point that is the point at which free residual chlorine will just be present under specific condition of time and temperature.

Apparatus:-

- Chlorine demand, bottles.
- Conical flasks, pipettes.

Reagent:-

- Sodium Thiosulphate N/35.5.
- Chlorine water.
- Starch.
- KI
- Acetic acid.

Procedure:-

- Place 200 ml of the well mix water sample in each of the 10 No.(250ml bottles).
- Add 0.5ml or required of standard chlorine water to the first bottle, then add standard Chlorine water in all 10 Nos. bottle in increasing order with fixed difference.
- Shake each bottle gently and allow to stand for 30minutes of contact period. After contact period add 1ml Acetic Acid in each bottle and shake gently then add a crystal of Potassium lodide and shake.
- Add 1ml of freshly prepared Starch solution in each bottle.

- Identify that bottle (out of 10 bottle), which contains the least blue color. The Chlorine water adds in this particular bottle may be recorded. Let it be x ml, that will be Chlorine demand of water.

The above value of chlorine demand up to break point chlorination, will be further increase by 0.2 to 0,3 mg/l for ensuring free chlorine to take care of subsequent contamination in the distribution system as to compute the total Chlorine demand of the water

Calculation:-

$$= \frac{\text{Raw Water x Chlorine Demand x 4.54}}{24}$$

= X kg/hour

JAR TEST

Apparatus:-

Jar Tester

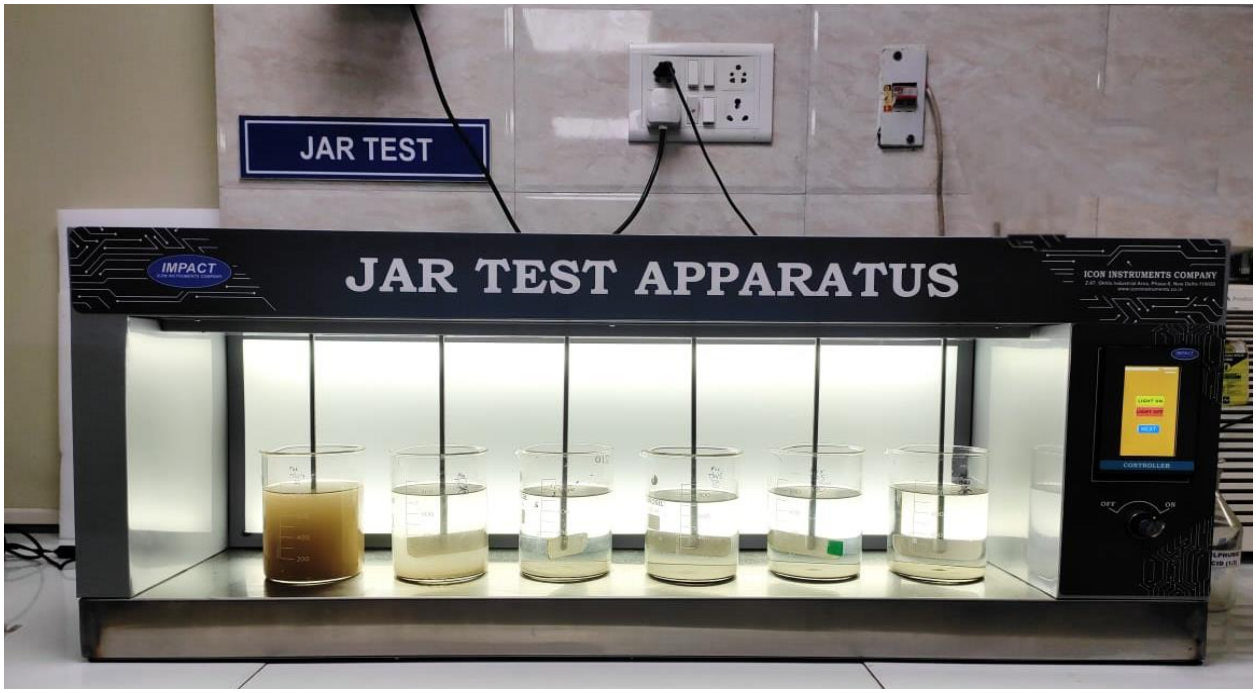
Reagents:-

1% Alum Solution:

1g alum dissolve in 100 ml distilled water mix well.

Procedure:-

1. Measure 1000ml of the sample into each of four beakers or six beakers and place them in multiple stirrer of jar tester.
2. Add graded doses of alum as possible to the beakers i.e. 1 ml(10ppm), 2ml(20ppm), 3ml(30ppm), 4ml(40ppm) etc.
3. Agitate for about 3 min at 200 RPM.
4. After 3 min agitate for 6-7 min at 20 RPM.
5. Leave them at rest for half an hour.
6. The test portion giving best flocculation, fast settlement and reduction of color and turbidity will indicate the optimum dose for alum.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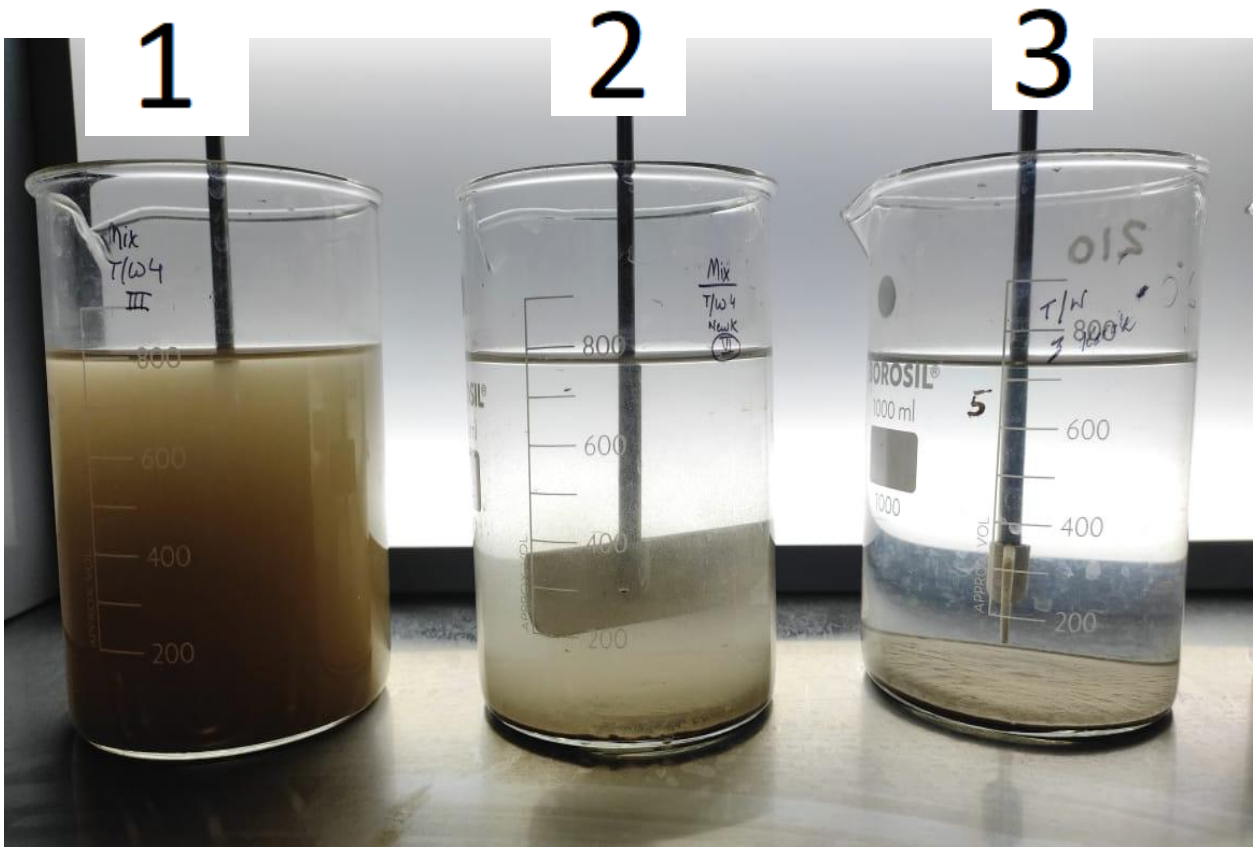

1
2
3
800
600
400
200
1000 ml

DISSOLVED OXYGEN (DO)

Scope:-

- Water and wastewater

Principle:-

The basic Winkler procedure entails the oxidation of manganese hydroxide In highly alkaline solution. Upon acidification in the presence of an lodide, the manganese hydroxide dissolve and free lodine is librated in amount equivalent to the oxygen originally dissolve in the sample the free lodine is titrated with a Sodium thiosluphate standard solution, using starch as internal indicator, after most of the lodine has been reduced. The normality of sodium thiosulphate is adjusted so that I ml is equivalent to 1 mg/l dissolved oxygen when 200 ml of the original sample is titrated.

Interference:-

There are various reducing and oxidising material, which can cause interferences. The alsterberg (sodium oxide) modification, which is used for most sewage, effluents, and streams especially if they contain more than 0.1 mg/1 nitrite and not more than 1 mg/l ferrous. Other reducing or oxidizing materials should be absent. If 1 ml potassium fluoride solution is added before acidifying the sample and there is no delay igration, the method is also applicable in the presence of 100-200 mg/l of ferric.

Apparatus:-

1. BOD bottles-300 mi capacity
2. Sampler

3. Volumetric flask

4. Conical flask

Reagents:-

1. Manganese Sulphate solution, MnSO4H2O: Weigh 364 g. MnSO4H2O and dissolved in DDW and make up to the I L. Filter the reagents if any sediment settles at the bottom of the flask.

2. Alkaline lodide-azide reagent:

Sodium hydroxide, NaOH - 500 g

Potassium lodide, Kl - 135 g

Sodium Azide, NaN3 - 10 g

Weigh the above mentioned quantilles of solution and transter to a 1 L volumetric flask. Dissolved NaN3, in 100 ml separately and add to alkaline KI solution. Make up the final volume to 1 L.

3. Sulphuric Acid. H_2SO4: concentrated.

4. Sodium Thiosulfate Stock Solution. Na2S2O3.5H2O, and [N/10]: Weigh 24.82 g Na2S2O3.5H2O. and transfer to I L volumetric flask. Dissolve in DDW, and make up to the mark.

5. Sodium Thiosulfate Titrant, Na2S2O3.5H2O. [N/40): Take 250 ml sodium thiosulfate stock solution to 1 L volumetric flask Dilute up to the mark with DDW.

6. Standard Potassium Dichromate. K2Cr2O7. [N/40]: Keep K_2CO; in an oven at 103C for 2 hr. Cool to room temperature in desiccators Weigh 1.226g dry, cool dichromate and transfer to a 1 L volumetric flask. Dissolve in DDW and dilute up to mark.

7. Potassium Iodide, KI: Take 2 g in a 500 ml Erlenmeyer flask and dissolve in 100 ml DDW

8. Starch Solution:

- Take 100 ml DDW in 250-ml beaker and keep on a heater equipped with magnetic stirrer
- Bring to boil.
- Weigh 2-g laboratory grade starch and suspend in a small volume of DDW.
- Add starch suspension in small increments to boiling DDW with constant stirring. Cool the solution. Add 0.2-g salicylic acid as preservative.

Standardization:-

Sodium Thiosulfate Solution

- Take 20 ml standard 0.025N K2Cr2O7. Solution and add 1 ml of 6N H_2SO. Place flask in dark.
- Dilute to 400 ml with DDW and titrate liberated iodine with thiosulfate.
- When pale straw color is reached, add few drops of starch indicator. A blue color will develop. Titrate t the blue color disappears. Record the reading.

$$\text{Normality of } Na_2S_2O3 = \frac{0.025 \times 20}{\text{Volume of Na2S2O3 used, ml}}$$

Procedure:-

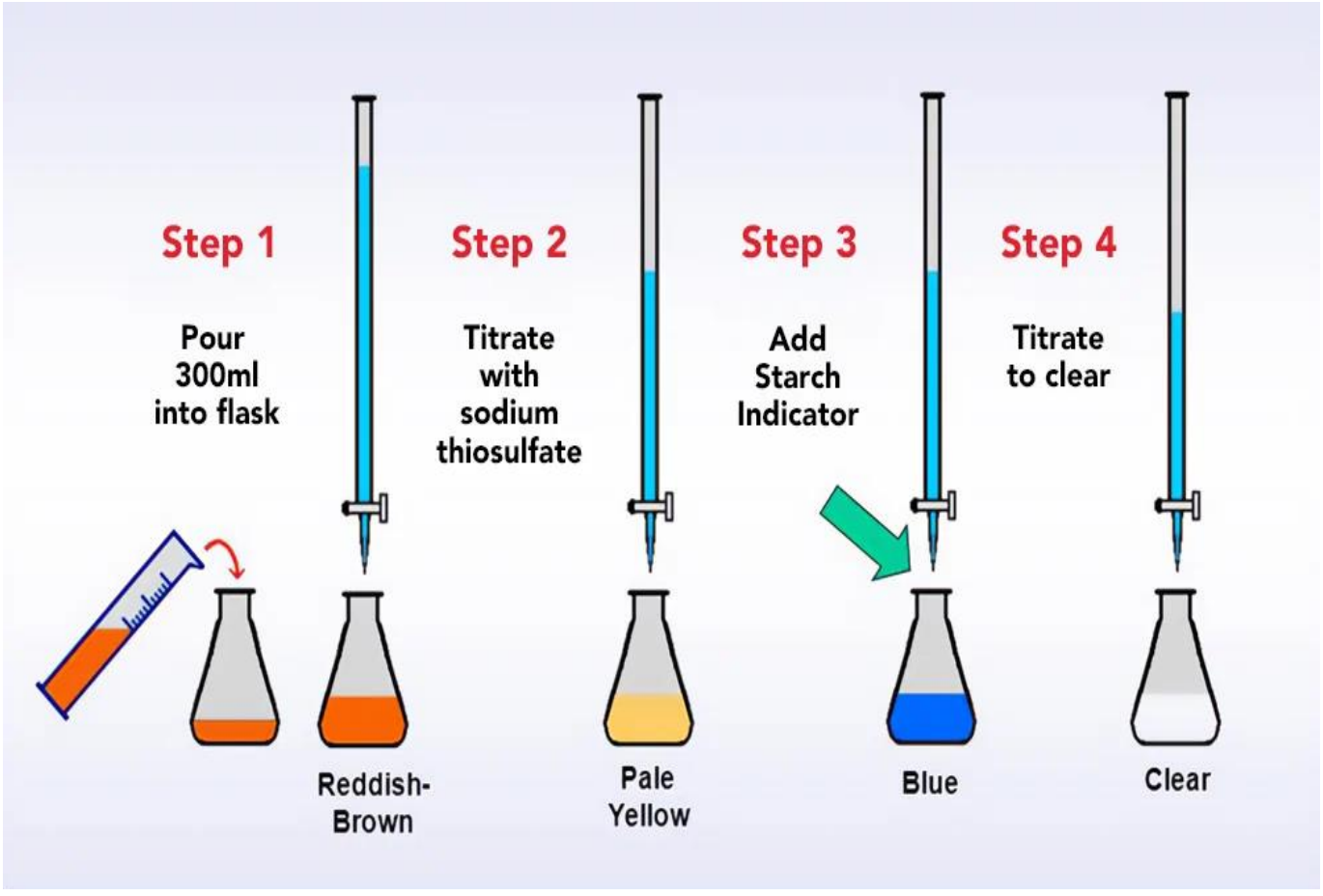
1. Take 300-ml BOD bottle and rinse it with sample. Pour the sample into the BOD bottle in such a way that it is allowed to overflow to avoid any entrapment of air bubbles. Close the bottle with stopper carefully.
2. Remove the stopper and add 2-ml MnSO4, reagent followed by 2-ml alkaline lodide-azide reagent Replace the stopper so that no air is trapped in the bottle. Pour any excess water off the bottle rim and invert it several times to mix. A brownish-orange flocculent precipitate will form, it sample contains DO. In absence of DO, a white precipitate will appear.

3. Allow the sample to stand until the flock has settled and left the top hall of the solution clear. Again invert it several times and let stand until the upper half of the bottle is clear. This is to ensure the complete reaction of chemicals with available DO.
4. Remove the stopper and immediately add 2-ml conc. H2SO4. Replace the stopper carefully to avoid the trapping any air bubbles. Invert several times to mix. The flock will dissolve and leave a yellowish-orange lodide color if DO is present.
5. Measure 204 ml of sample, which corresponds to 200-ml of the original sample(correction for the sample loss by displacement with reagent) into a 250-ml conical flask.
6. Titrate this solution with standard Na2SO35H_2O-[N/40) solution to faint yellow color. Add 1-2 drop of starch indicator and swirl to mix. A dark blue color is developed.
7. Continue the titration until the solution change from blue to colorless.

Calculation:-

1.0ml of $Na_2S2O3.5H2O$. [N/40]= 1 mg DO

So, the ml solution of $No_2SO3.5H2O$. (N/40) used is equal to the mg/l of DO available in the sample.



OXYGEN ABSORPTION-3 HR. AT 37 °C

Scope:-

Water and wastewater

Principle:-

When potassium permanganate reacts with sulphuric acid it gives the dipotassium sulphate and MnSO4, wafer and oxygen radicals.

$$2\ KMnO4 + H_2SO4 \rightarrow K2SO4 + 2MnSO4 + 3H2O + 5O$$

The free oxygen oxidized the organic matter present in the sample.

Organic matter + 5 $0 \rightarrow CO2 + H2O$

The remaining oxygen present in the sample can be measured by adding the KI solution, which liberate the equal amount of the lodine, which is titrated with the sodium thiosulfate solution in the presence of starch.

$$KI + O \rightarrow 12 \text{ (form blue color with starch)}$$

$$2\ Na_2SO3 + I_2 \rightarrow Na_2S4O6 + 2\ Nal$$

Apparatus:-

1. BOD bottles-300 ml capacity with stopper
2. Incubator-set at 37 OC
3. Dispenser
4. Digital burette
5. Volumetric flask

Reagents:-

1. Potassium Permanganate Stock Solution, KMnO.10.1 N]: Weigh 3.2 g KMnO and transfer in 1 L volumetric flask and swirl the flask to dissolve and make up the mark with DDW
2. Standard Potassium Permanganate Solution, KMnO4(0.025N): Take 250 ml stock KMnO, in 1L volumetric flask and make up to the mark with DDW.
3. Sulphuric Acid, H_2SO4, [1:3]: Take 500 ml DDW in volumetric flask and slowly add 250 ml conc. H2SO4 and cool to room temperature.
4. Sodium Thiosulfate Stock Solution, Na2S2O3.5H2O, and [N/10): Weigh 24.82 g Na2S2O3.5H2O, and transfer to 1 L volumetric flask. Dissolve in DDW, and make up to the mark.
5. Sodium Thiosulfate titrant, Na2S2O35H2O, (N/40): Take 250 ml sodium thiosulfate stock solution to 1 L volumetric flask. Dilute up to the mark with DDW.
6. Standard Potassium Dichromate, K2Cr2O7, [N/40): Keep K2Cr2O7 in an oven at 103C for 2 hr. Cool to room temperature in a desiccators Weigh 1.226 g dry, cool dichromate and transfer to a 1 L volumetric flask. Dissolve in DDW and dilute up to mark.
7. Potassium Iodide, KI: Take 2 g in a 500 ml Erlenmeyer flask and dissolve in 100 ml DDW.
8. Starch Solution:
    - Take 100 ml DDW in 250-ml beaker and keep on a heater equipped with magnetic stirrer.
    - Bring to boil,
    - Weigh 2-g laboratory grade starch and suspend in a small volume. of DDW.
    - Add starch suspension in small increments to boiling DDW with constant stirring. Cool the solution. Add 0.2-g salicylic acid on preservative.

Standardization:-

a. Sodium Thiosulfate Solution

1. Take 20 ml standard 0.025N K_2Cr_2O7 Solution and add 1 ml of 6N H_2SO4. Place flask in dark.
2. Dilute to 400 ml with DDW and titrate liberated iodine with thiosulfate.

BACTERIOLOGICAL LAB

CLEANING OF GLASSWARES

- Sampling bottles, culture tubes, flask, and glass Petri dishes should always be cleaned with Sodium carbonate, rinsed in running tap water, and then in distilled water. Finally, the cleaned and washed glassware should be sterilized in hot air oven at 160 °C for one hour.

- Add 0.5ml of 5% thiosulphate solution in sampling bottle and put a thin strip of paper between the stopper and the neck of the bottle. The neck and stopper of bottles should be covered with paper or foil before sterilization.

- Pipettes used for biological transfers should be placed in a jar of disinfectant having free chlorine after every use. They must be thoroughly washed with fresh water and finally rinsed with distilled water. Dry and pack them in a metal canister and sterilize .After sterilization, plug the pipettes with cotton wool.

STORAGE OF PREPARED MEDIA

- Broth media in culture tubes or flaks should be provided with air tight seal.
- Agar containing media should not be held at higher temperature (40 to 50 °C) for longer time as agar tends s to clump.
- Agar plate should be stored at 2 to 8 °C in sealed conditions in containers to avoid loss of moisture.
- If not otherwise specified under controlled conditions, media should be stored at 12 to 15 °C.
- Media should not be stored below 0 °C
- Unless the stability of the prepared medium is known it should not be stored for longer periods

TOTAL COLIFORM TEST

This test is used for estimation of number of the coliform groups which includes the entire aerobic and facultative anaerobic gram negative, non spore forming, rod shaped bacteria.

The test shall be carried out by either of the two method

1. Multiple tube dilution method.
2. Membranes filter method.

A. MULTIPLE TUBE DILUTION METHOD

In this method, different volumes of undiluted or diluted samples are added to a series of tubes containing specific differential broth medium. After incubation, certain tubes show the characteristic changes of color due to the growth of required organism which are considered as a positive reaction tubes. Tubes showing no color change are considered negative reaction tube.

APPARATUS:-

Sampling bottles, pipettes, pipette container, Culture tubes, Durham's tubes, non absorbent cotton, incubator, serological water bath, autoclave.

MEDIA:-

Dehydrated MacConkey Broth, Dehydrated BGB, Peptone water, Dehydrated MacConkey agar, dehydrated nutrient agar.

REAGENT:- Dilution water, Kovac Indol reagent.

STANDARDIZATION:- Standardization of the process is not required. However, a positive and negative control should be run to ascertain respectively the quality of the medium and the accuracy of the technique used-

- Positive Control: Use a pure culture in place of sample. Transfer aseptically the pure culture cells in a culture tube containing growth medium.
- Negative Control: Use sterile dilution water in place of sample as a negative control.
  Control must be run with every stage in this method.

PROCEDURE:- This method is comprised of following completely independent tests namely-

1. Presumptive test
2. Confirmed test.
3. Complete test.

Presumptive Test:-

1.1 Application to polluted water:

- Arrange five rows of three tubes each in a test tube rack. The tubes in the first row (F1) hold 10 ml of double strength MacConkey broth while other rows (F2, F3, F4, F5) contain 10 ml of single strength MacConkey broth.
- With a sterile pipette add 10 ml of sample, after proper mixing, to each of the three tubes in row FI.
- With a sterile pipette, add 1 ml of sample to each of the three tubes in row F2.
- Prepare a 1:10 dilution of the sample by adding 1 ml of sample to 9 ml of dilution water. Prepare progressive dilution for F4 & F5.
- With sterile pipettes add 1 ml of the respective dilution to each of the three tubes in respective rows F3, F4 & F5.
- After gently shaking the tubes to mix the inoculums, incubate the rack at 37 °C for 24-48 hours.
- Record the positive (color change with gas in Durham tube) and negative tubes after 24 & 48 hours. (h) Determine the MPN on the readings of 48 hours.(Refer to appropriate MPN table in Annexure 1)

1.2 Application to treated water:

1. Arrange one row of five tubes in a test tube rack. The tubes hold 10 ml of double
2. With a sterile pipette add 10 ml of sample to each of the tubes in row.
3. After gently shaking the tubes to mix the inoculums, incubate the rack at 370C strength MacConkey broth...for 24-48 hours.
4. Record the positive (color change with gas in Durham tube) and negative tubes after 24 & 48 hours.
5. Determine the MPN on the readings of 48 hours. (Refer to appropriate MPN table in Annexure II).

   Subculture all presumptive positive tubes of the Coliform test at the end of 24 and 48 hours into two set of BGB medium. Incubate one set at 44.5 °C for 24 hr. in water bath for faecal coliform test and other set for confirmed test.

FAECAL COLIFORM TEST

This test is used to differentiate coliforms of faecal origin from those of non faecal origin. The test shall be carried out by either of the two methods:

1. Multiple tube dilution method
2. Membrane filter method

A. MULTIPLE TUBE DILUTION METHOD

APPARATUS:

Sampling bottles, pipettes, pipette container, metallic loop serological water bath, culture

tube.

MEDIA:-

Dehydrated Brilliant Green Bile lactose Broth (BGB). Media should be prepared in accordance with the manufacturer's instructions)

STANDARDIZATION:-

Standardization of the process is not required. However, a positive and negative control should be run to ascertain respectively the quality of the medium and the accuracy of the technique used-

- Positive Control: Use pure culture in place of sample. Transfer aseptically the pure culture cells in a culture tube containing BGB medium.
- Negative Control: Use sterile dilution water in place of sample as a negative control.

PROCEDURE:-

1. Subculture all presumptive positive tubes of the coliform test at the end of 24 and 48 hours into tube containing 5 ml BGB medium and incubate at 44.5 °C for 24 hr. in water bath.
2. Counting: Gas formation within 24 hr. is considered a positive reaction for faecal coliform

CALCULATION:-

The positive and negative tubes in various combinations shall be used for calculating most probable number (MPN) of bacteria with help of MPN table. The result shall be expressed as MPN/ 100 ml (Refer Annexure 1).

B. MEMBRANE FILTER METHOD:

PRINCIPLE:

Filter a volume of water sample through a sterile 0.45um membrane filter. Place the membrane filter on an M FC- agar medium. Incubate the dishes at 44.5°C for 24 hours. Count all blue colored colonies as faecal colonies.

APPARATUS:

Sampling bottles. Petri dishes, pipettes, pipette container, volumetric flask (100ml, 250ml). Membrane filtration unit, colony counter, serological water bath, disposable bags. membrane filters (47mm diameter, pore size 0.45pm)

MEDIA: Dehydrated M- FC Agar (media should be prepared in accordance with the manufacturer's instructions).

REAGENT:-

1% Rosalic acid

SELECTION OF SAMPLE SIZE:-

Take the sample volume (without or with dilution) that should result in growth of 20 colonies and not more than 200 colonies of all types. Always filter the sample in duplicate

STANDARDIZATION:- Standardization of the process is not required. However, a positive and negative control should be run to ascertain respectively the quality of the medium and the accuracy of the technique used-

a. **Positive Control:** Use a pure culture in place of sample. Transfer aseptically the pure culture cells in a culture tube containing 10 ml sterile dilution water. Follow the same procedure of filtration and incubation as described below for the sample.
b. **Negative Control:** Use sterile dilution water in place of sample as a negative control.

PROCEDURE:

- Filtration of Sample: Using sterile forceps, place a sterile membrane filter over the stainless steel mesh of the apparatus, grid side up. Place the funnel unit carefully over the receptacle and lock it in place. Then pass the sample through the filter under

vacuum. Rinse the filter by filtration 2 to 3 times with 20 to 30 ml of sterile buffer water Unlock the assembly, remove the filter by sterile forceps and place it on the sterile agar plate with rolling motion to avoid the entrapment of air.

- Invert the agar plate and incubate at 44.5 OC for 24 hr.
- Count all blue colonies with the help of stereo microscope.

CALCULATION:-

$$\text{Faecal coliform/100 ml} = \frac{\text{No, of colonies counted x 100}}{\text{Volume of sample filtered}}$$

If any dilution is done, consider the dilution factor (df) and calculate the colonies with the following relation:

$$\text{Faecal coliform/100 ml} = \frac{\text{No of colonies countedx df x 100}}{\text{Volume of sample filtered}}$$

DISPOSAL: The agar plate with bacterial colonies must be autoclaved at 121 0 C (15 psi) for 15 minutes and disposed of in disposable bags. The petridish must be clean and disinfected.

E. COLI TEST

E. coli is one of the members of faecal coliforms and chief indicator of faccal contamination. This bacterium can be identified and confirmed by the IMVIC series of tests representing Indole, Methyl red, Voges Proskauer and Citrate tests,

A. INDOLE TEST:

Principle:-

Oxidation of tryptophan to indole by the action of tryptophanase enzyme is the basic principle of this test. The presence of indole is detected by Kovac's reagents which produce a cherry red color.

Apparatus:-

Inoculation needle, Bunsen burner

Media:-

Tryptone broth.

Reagent:-

Kovac indole reagent.

Procedure:

a. Subculture from all the positive tubes of BGB broth at 44.50C into tubes of tryptone water.
b. Incubate all the inoculated tubes at 370C for 24 hour.
c. After 24 hour of incubation add 0.2-0.3 ml of Kovac's reagent and shake the tubes gently. Record the color change.

Test Confirmation

(a) Cherry red color in the amyl alcohol layer- positive indole

(b) Retention of original color of the reagent- negative indole

E. coli gives positive test.

B.METHYL RED TEST:

Principle:-

Glucose is the major substrate oxidized by all enteric organisms for energy production. In this biochemical oxidation, different organic acids are produced as end products. The pH indicator methyl red detects the presence of acids. It turns red at pH 4.0, which is an indication of a positive test. At pH 6.0, though it is an acidic condition, the indicator turns yellow because of lower H+

concentration. This indicates a negative test.

Apparatus:-

Inoculation needle, Bunsen burner

Media:-

Glucose phosphate broth

Reagent

Methyl red reagent.

Procedure

Subculture from all the positive tubes of BOB broth at 44.5 into tubes containing 10 ml volumes of glucose phosphate broth b. Incubate all the inoculated tubes at 370C for 48 hour c. After 48 hour of incubation add 10 drops of methyl red indicator and shake the tubes gently. Record the color change.

Test Confirmation

- Red color- positive methyl red
- Yellow color-negative methyl red
- E. coli gives positive test.

CVOCES PROSKAUER TEST

Principle:

Some organism produces nonacid or neutral end products which are detected by alkaline a-napthol solution (Barritt's reagent).

Development of deep rose red color in the culture after 15 minutes following the addition of Barritte's reagent indication of positive test. The absence of red rose calor development is the negative test

Apparatus:

Inoculation needle, Bunsen burner

Media: Glucose phosphate broth.

Reagent: Barritte's reagent, 40% w/v) KOH

Procedure:

a. Take two culture tubes inoculated in methyl red test. b. Incubate the inoculated tubes at 350C for 48 hour

c. Add 3 ml a-napthol solution and 1 ml KOH solution and shake vigorously. Record the color change.

Test Confirmation:

- Rose red color-positive Voges Proskauer
- No color development-negative Voges Proskauer

E. coli gives negative test.

D.CITRATE TEST

Principle

Some enteric organisms are able to use citrate as their sole carbon source for energy in the absence of glucose or lactose. Citrate is acted on by the enzyme citrase and produces some alkaline products that change the color of Bromothymol blue indicator from green to deep Prussian blue. Apparatus: Inoculation needle, Bunsen burner

Media:

Simmons citrate agar.

Reagent:

4% NaOH, Bromothymol blue indicator

Procedure:

- Inoculate the slants of Simmons citrate with culture from the positive tubes of BGB broth at 44.50C.
- Incubate all the inoculated tubes at 370C for 24-48hour.

Test Confirmation:

a. Growth with Prussian blue color- positive test.
b. No growth with green color of thymol indicator- negative test

E. coli gives negative test.