ENVIRONMENTAL ENGINEERING LABORATORY MANUAL

2014-2015

Code: 40131

MALLA REDDY ENGINEERING COLLEGE
(Autonomous)

IV YEAR I SEMESTER

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ENVIRONMENTAL ENGINEERING LAB

Course Objectives:

- Ability to apply knowledge of mathematics and engineering in the calculation of Characteristics of water and waste water(pH, Acidity, Alkalinity, Total solids, Dissolved oxygen, BOD, COD etc)
- Understanding of professional and ethical responsibility in the areas of testing
- Ability to communicate effectively the charcteristics of samples.
- The broad education to understand the impact of engineering solutions in a global and societal context with respect to problems
- Ability to life-long learning with the advances in testing.

LIST OF EXPERIMENTS

1. Determination of pH and Turbidity
2. Determination of Conductivity and Total dissolved solids (Organic and Inorganic)
3. Determination of Alkalinity/Acidity.
4. Determination of Chlorides.
5. Determination of iron.
6. Determination of Dissolved Oxygen.
7. Determination of Nitrates.
8. Determination of Optimum dose of coagulant
9. Determination of total Phosphorous.
10. Determination of B.O.D
11. Determination of C.O.D
12. Determination of Optimum coagulant dose.
13. Determination of Chlorine demand.
14. Presumptive coliform test.

NOTE: At least 8 of the above experiments are to be conducted.

Course Outcomes:

- Ability to analyze the water and waste water samples and classify them.
- Ability to identify the potable water.
- Ability to provide the type of treatment required.

2015 -16

Malla Reddy Engineering College (Autonomous)

L T P
- - 4
Credits: 2

Course Code: 50117

B.Tech. – V Semester
ENVIRONMENTAL ENGINEERING LAB

LIST OF EXPERIMENTS:

1. Determination of pH and Turbidity
2. Determination of Conductivity and Total dissolved solids (Organic and Inorganic)
3. Determination of Alkalinity/Acidity.
4. Determination of Chlorides.
5. Determination of iron.
6. Determination of Dissolved Oxygen.
7. Determination of Nitrates.
8. Determination of Total Hardness.
9. Determination of Sulphates.
10. Determination of B.O.D
11. Determination of C.O.D
12. Determination of Optimum coagulant dose.
13. Determination of Chlorine demand.
14. Presumptive coliform test.

ENVIRONMENTAL ENGINEERING LABORATORY SPECIFICATIONS

APPENDIX I

WATER QUALITY STANDARDS (CPHEEO)

PHYSICAL AND CHEMICAL STANDARDS

S. No.	Characteristics	*Acceptable	**Cause for Rejection
1	Turbidity (Units on J.T.U. Scale)	2.5	10
2.	Colour (Units on Platinum Cobalt Scale)	5.0	25
3	Taste and Odour	Unobjectionable	Unobjectionable
4	pH	7.0 to 8.5	<6.5 or > 9.2
5.	Total Dissolved Solids (mg /L)	500	1500
6	Total Hardness (mg/L) (ad $CaCO_3$)	200	600
7.	Chlorides (as Cl) (mg/L)	200	1000
8.	Sulphates (as SO_4)	200	400
9.	Fluorides (as F) (mg /L)	1.0	1.5
10.	Nitrates (as NO_3) (mg /L)	45	45
11.	Calcium (as Ca) (mg /L)	75	200
12.	Magnesium (as Mg) (mg/L)	30	150
	if there are 250 mg/L of sulphates, Mgcontent can be increased to a maximum of 125 mg/l with the reduction of sulphates at the rate of I unit per every 2.5 units of sulphates.		
13.	Iron (as Fe) (mg/L)	0.1	1.0
14.	Manganese (as Mn) (mg/L)	0.05	0.5
15.	Copper (as Cu) (mg/L)	0.05	1.5
16.	Zinc (as Zn) (mg/L)	5.0	15.0
17.	Phenolic Compounds (as Phenol) (mg/L)	0.001	0.002
18.	Anionic Detergents (mg/L) (as MBAS)	0.2	1.0
19.	Mineral Oil (mg/L)	0.01	0.3

S. No.	Characteristics	*Acceptable	**Cause for Rejection
	TOXIC MATERIALS		
20.	Arsenic (as As) (mg/L)	0.05	0.05
21.	Cadmium (as Cd) (mg/L)	0.01	0.0
22.	Chromium (as Hexavalent Cr) (mg/L)	0.05	0.05
23.	Cyanides (as CN) (mg/L)	0.05	0.05
24.	Lead (as Pb) (mg/L)	0.1	0.1
25.	Selenium (as Se) (mg/L)	0.01	0.01
26.	Mercury (total as Hg) (mg/L)	0.001	0.001
27.	Polynuclear Aromatic Hydrocarbons (PAH)	0.2 µg/L	0.2 µg/L
	RADIO ACTIVITY		
28.	Gross Alpha Activity	3_p Ci / L	3_p Ci /L
29.	Gross Beta Activity	30_p Ci / L	30_p Ci / L

NOTES :

*1. The figures indicated under the column 'Acceptable' are the limits up to which water is generally acceptable to the consumers.

*2. Figures in excess of those mentioned under 'Acceptable render the water not acceptable, but still may be tolerated in the absence of alternative and better source but up to the limits indicated under column "Cause for Rejection" above which the supply will have to be rejected.

*3. It is possible that some mined and spring waters may exceed these ratio activity limits and in such cases it is necessary to analyse the individual radio nuclides in order to assess the acceptability or otherwise for public consumption.

GUIDELINE VALUES FOR BACTERIOLOGICAL QUALITY

Organism	Unit	Guideline Value	Remarks
A.	Piped Water Supplies		
A.1	Treated water entering the distribution system		
	Faecal coliforms number /100 ml	0	turbidity < 1 NTU
	Coliform organisms number/100 ml	0	for disinfection with chlorine, pH preferably 8.0, free chlorine residual 0.2-0.5 mg/litre following 30 minutes (minimum) contact.
A.2	Untreated water entering the distribution system		
	Faecal coliforms number/100 ml	0	
	Coliform organisms number/100 ml	0	in 98% of samples examined throughout the year in the case of large supplies when sufficient samples are examined.
	Coliform organisms number / 100 ml	3	in an occasional sample, but not in consecutive samples.
A.3	Water in the distribution system		
	faecal coliforms number/100 ml	0	
	Coliform organisms number / 100 ml	0	in 95% of samples examined throughout the year in the case of large supplies when sufficient samples are examined.
	Coliform organisms number / 100 mL	3	in an occasional sample but not in consecutive samples.
B.	Unpiped water supplies		Should not occur repeatedly. If
	Faecal coliforms number / 100 mL	0	occurence is frequent and if sanitary
	Coliform organisms number / 100 mL	10	protection cannot be improved an alternative source must be found, if possible
C.	Emergency water supplies		advise public to boil water in case of
	Faecal coliforms number / 100 ml	0	failure to meet guideline values
	Coliform organisms number / 100 ml	0	

Source : Guidelines for Drinking Water Quality Volume 1, a W.H.O. Publication.

DETERMINATION OF P^H

EXPERIMENT-1 (a)

AIM

To determine the pH of the given sample using the pH meter.

APPARATUS

PH meter

REAGENTS

Standard buffer solution.

THEORY

The pH value is a measure of the acidity or alkalinity of a solution. It is expressed as the negative logarithm of hydrogen ion concentration of the solution.

$pH = - \log [H^+]$

Where $[H^+]$ = Hydrogen ion concentration of the solution in moles per liter.

The pH scale is used to express the degree of acidity or alkalinity. The pH scale extends from 0 to 14 with 7 as the pH of pure water at 25^0C taken as the neutral point. pH greater than 7 and upto 14 indicates the degree of alkalinity, and pH less than 7 and upto 0 indicates the degree of acidity.

PRINCIPLE

When the pair of electrodes namely the pH sensitive glass electrode and reference electrode are dipped in a solution, they develop an electrical potential, which is also dependent on the temperature of the solution. This electrical potential is calibrated and read as pH on the indicator.

PROCEDURE

1. Clamp the glass and reference electrode to the electrode stand and connect them to the pH meter appropriately.

2. Insert the plug in to 220V A.C.Mains. Switch on the instrument by turning the power ON switch. Keep the instrument in the stand by position and wait for a few minutes till the instrument gets warmed up.
3. Prepare the buffer solution by dissolving the buffer substance supplied in fresh distilled water.
4. Take the buffer solution in a clean glass beaker. Wash the electrode with distilled water and clean with tissue paper. Lower the electrodes in the solution.
5. Measure the temperature of the solution and set the temperature compensator to this value. See the meter pointer to read 7 pH exactly by means of "Set 7" control. Keep the instrument in the proper pH range. Set the pointer to the known pH value of the buffer solution by turning the calibrate control.
6. To measure the pH value of the given solution, wash the electrode with distilled water and clean with filter paper. Take the solution in a clean glass beaker and immerse the electrodes in it.
7. Measure the temperature of the solution and adjust the temperature compensator to that value. Set the range control in the expected range. Turn the control from stand by to READ position. The reading shown in the appropriate scale of the meter is the pH value of the given solution.

OBSERVATIONS

S. No.	Temperature	pH		Type of sample
		pH meter	pH paper	

RESULT

PH of the given sample of water = ----------

PRECAUTIONS

1. The instrument should be kept in the stand by position only, unless during the actual measurement.
2. "Set 7" control should be operated only in the stand by position.

3. While cleaning the electrodes, do not touch the tip of the electrodes.

IMPORTANCE

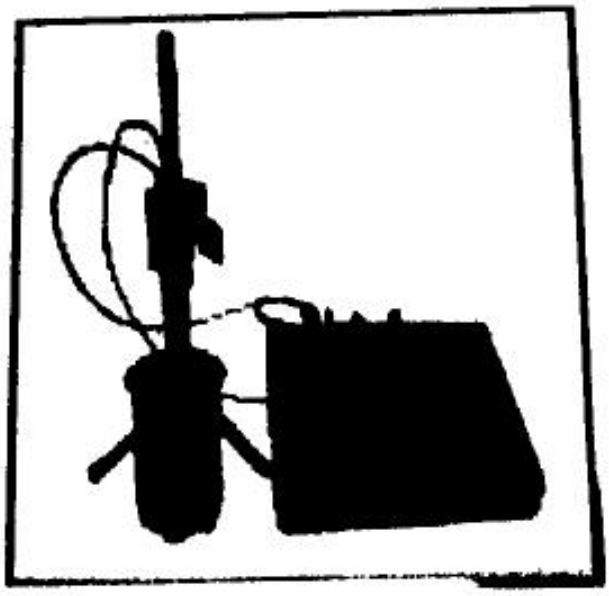
1. In the water treatment, pH is a factor to be considered in chemical coagulation, disinfection, and water softening also in corrosion control, because in these processes, certain reactions will take place only in the proper pH ranges.
2. In the wastewater treatment involving biological processes, pH must be controlled within a range favorable to the particular organisms involved. Chemical coagulation of wastewater's, de-watering of sludge, oxidization of wastes require the pH to be controlled in the favorable range.
3. For public water supplies, pH value should preferably be between 6.5 and 8.5.

Viva questions

- What is the measure of hydrogen ion concentration
- pH of natural water?
- Acidic water has pH below 7
- Basic water has pH above 7
- Desirable limit 6.5-8.5

ISSUES:

Beyond this limit the water will affect the mucous membrane and water supply system



TURBIDITY

EXPERIMENT-1 (b)

AIM

To determine the turbidity of the given sample using Nephelo – Turbidity meter.

APPARATUS

1. Nephelo – Turbidity meter with sample cell,
2. Cell riser and light shield.

REAGENTS

Standard FORMAZIN solutions.

THEORY

Turbidity of water is a term, which is applied to waters containing suspended matter that interferes with passage of light through it. It is expressed by the amount of suspended matter in parts per million (mg/l), in water as determined by optical observations.

PRINCIPLE

Nephelo-Turbidity meter type 131 operates on the principle that light passing through a substance is scattered by matter suspended in the substance. In this instrument, a strong light beam is passed upward through a tube containing the sample. As the beam passes through the sample, the light is scattered in proportion to suspended particles. At 90^0 to the light path, this scattered light is sensed by the phototube to give the turbidity reading. The unit of measurement is Nephelometric Turbidity Unit (NTU).

PROCEDURE

1. Insert the three-pin plug in the mains and switch on the power switch on the front panel and wait for about 15 minutes.
2. Standardize instrument with the help of standard solutions of 1 NTU, 10 NTU, 100 NTU and 500 NTU respectively.
3. Set the range switch in the 0 – 1000 NTU range.
4. Insert the cell riser in the cell compartment, keep the sample cell filled with distilled water up to the mark indicated in the cell compartment and cover it with light shield.
5. Adjust the pointer to read zero on the panel meter using SET ZERO control.
6. Remove the distilled water and take 500 NTU solutions into the sample cell and put it in the cell compartment and cover it with light shield.
7. Adjust standardize control to read 50 on the scale.
8. Remove cell riser, put a cell filled with distilled water in the cell compartment and close it with light shield.
9. Standardize with 1 NTU, 10 NTU, 100 NTU solutions for the 0-1, 0-10, 0-100 NTU ranges in the same procedure.
10. Clean the cell with distilled water.
11. Take the sample into the cell upto the mark indicated.
12. Put the cell into the cell compartment and close it with light shield.
13. Adjust the range switch in the appropriate position.
14. Note down the panel meter reading which directly indicates turbidity in NTU.

OBSERVATIONS

S. No.	Turbidity of the sample (NTU)

RESULT

Turbidity of the given sample of water = NTU

PRECAUTIONS

1. Use cell riser only for 0-1000 NTU range.

2. Use light shield to close the sample cell.
3. **DO NOT** calibrate 0-1000 NTU range with 1000 NTU standard solution.

NOTE: PREPARATION OF STANDARD SOLUTION

To prepare the standard solutions a substance known as FORMAZIN is used.

1. Dissolve 5g of Hydrazine sulphate in 400 ml of distilled water.
2. Dissolve 50g of pure Hexamethylene Tetramine in 400ml of distilled water.
3. Add both solutions in 1-liter volumetric flask and add distilled water to make this mixture to 1 liter.
4. This mixture is allowed to settle for 48 hours at normal room temperature. During this time it will develop suspension particles. Before preparing further standard solutions, shake the flask thoroughly.

The following table gives the method of preparation of standard solutions.

N.T.U	FORMAZINE in ml. diluted to 1 liter with turbidity free water	FORMAZINE in ml. diluted to 100 liters with turbidity free water
500	125.00	12.50
100	25.00	2.50
10	2.50	0.25
1	0.25	-

IMPORTANCE

1. **The presence of turbidity in drinking water will make the consumer hesitate to take such water. It is also an indication of possible contamination with impurities and wastewater, which is harmful to consumers. The turbidity of drinking water shall not exceed 10 mg/l on the Silica Scale.**
2. **If the turbidity of water is more, filtration of water becomes more difficult and costly. For high turbid water the use of slow sand filters are impracticable. Satisfactory operation of rapid sand filters depend upon the effective removal of turbidity before the water is admitted to the filter.**

3. The effectiveness of disinfection is reduced by the presence of turbidity in the water. These turbid particles protect harmful bacteria and do not allow the chemicals to come in direct contact with bacteria.

Viva Questions:

1. What is turbidity?
2. Why turbidity is caused in water?
3. What is the range of permissibility for drinking water?
4. What are the effects of turbid water?
5. Units of turbidity?

ISSUES:

- High level turbidity shield and protect bacteria from the action of disinfecting agents
- Desirable limit-5 NTU
  should be below 1 NTU when disinfection is practiced
  Permissible limit-10 NTU

In picture: 5, 50, 500 NTU

CONDUCTIVITY METER

EXPERIMENT-2 (a)

AIM

To determine the specific conductivity of the given sample.

APPARATUS

Digital conductivity meter

REAGENTS

Standard KCl Solution.

THEORY

The specific conductivity of the water is its ability to conduct electricity. It is determined by measuring the conductivity of water at 25^0 C. The unit of specific conductivity is micro siemens per Cms (μs/Cm) or micro-mhos per Cm (μmhos/Cm).

PRINCIPLE

The digital conductivity meter measures the specific conductivity by employing the wheat-stone bridge principle. The cell and temperature probes of the instruments are dipped into the given sample to find the specific conductivity of the given sample. The specific conductivity multiplied by a conversion factor gives the total dissolved solids.

PROCEDURE

1. Connect the conductivity cell and temperature probe to the socket 'CELL' and 'TEMP' respectively provided on instrument.
2. Connect the mains cord to 230V, 50 Hz supply.
3. Dip the cell and temperature probe in a beaker containing a solution of whose specific conductivity is known.
4. Depress the red button. Immediately the display will read some value
5. Depress the button labeled 'TEMP'. Now that the display will read the temperature of the solution.
6. Set the measured temperature on the temperature compensation switch.

7. Depending on the specific conductivity of the solution taken, select and depress the button.
8. Adjust the cell constant adjustment knobs using a screwdriver until the display reads the specific conductivity of the solution. Now the instrument is calibrated. Once the instrument is calibrated it is not necessary to repeat this step every time the instrument is used.
9. Rinse the cell in distilled water and dip it in beaker containing the unknown solution. Depress the button in descending order until the display reads the specific conductivity of the sample given.
10. The display directly gives the specific conductivity of the given sample is μs/Cm.
11. Total dissolved solids present in the given sample are calculated by multiplying the specific conductivity with conversion factor (0.65).
12. The specific conductivity is multiplied with conversion factor (0.65) to get total dissolved solids present in the given sample in mg/l.

OBSERVATIONS

S. No.	Temperature	Conductivity (μs/Cm)

RESULT

The specific conductivity of the given sample = μs/Cm

Total dissolved solids present in the given sample = mg/l

Viva Questions:

1. Why do water conducts electricity?
2. When water shows more conductances what does it indicates?
3. How conductance are measured in water
4. What is the permissible limits of water?

TOTAL DISSOLVED SOLIDS

EXPERIMENT-2 (b)

AIM

To determine the total dissolved solids of the given sample.

APPARATUS

1. Evaporating Dishes
2. Oven
3. Desiccators
4. What man filter paper No.44
5. Water bath

PRINCIPLE

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

PROCEDURE

1. A clean porcelain dish is ignited in a muffle furnace and after partial cooling in the air, it is cooled in a desiccators and weighed.
2. A 100 ml of filtered sample is placed in the dish and evaporated at 100^0 C on water bath, followed by drying in over at 103^0 C for 1 hour.
3. Dry to a constant weight at 103^0 C, cool in a desiccators and weigh.

OBSERVATIONS

S. No.	Volume of sample (ml)	Initial weight of the dish (mg)	Final weight of the dish (mg)	Total solids (mg/l)

CALCULATIONS

$$\text{Total solids} = \frac{(W_2 - W_1) \times 1000}{\text{ml of sample taken}} \quad \text{mg/l}$$

W_1 = Initial weight of the dish in mg.

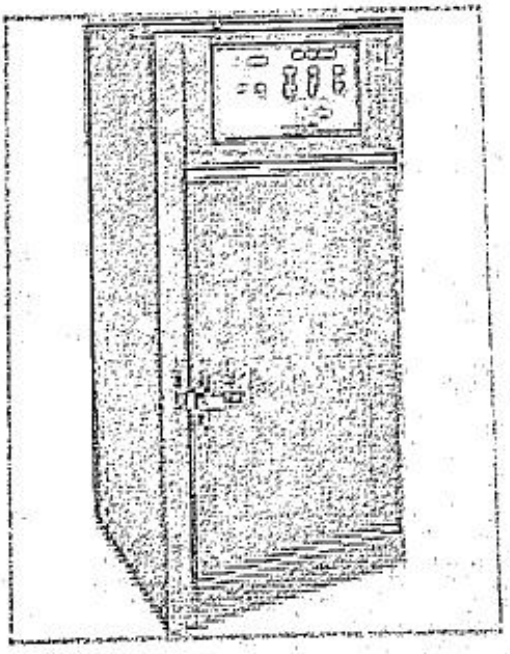
W_2 = Final weight of the dish in mg.

RESULT

Total dissolved solids of the given sample = mg/l

Viva Questions:

1. What are Dissolved solids?
2. How do they contaminate in water?
3. What is the permissible limit for drinking water?
4. What are the consequences when they are beyond the limit?



pink with the addition of Methyl orange indicator will show total alkalinity, i.e., OH^-, CO_3^{--} and HCO_3^-.

pH	0	4.3	8.3	14.0
Indicator		Methyl orange	Phenolphthalein	

INTERFERENCE

Colour, turbidity, iron, aluminium and residual chlorine are prime sources of interference.

PROCEDURE

1. Take 20ml sample in a conical flask and add 2-3 drops of Phenolphthalein indicator.
2. If pink color develops titrate with 0.02 N H_2SO_4 till it disappears indicating pH 8.3. Note the volume of H_2SO_4 required (A ml).
3. Add 2-3 drops of Methyl orange to the same flask. The sample turns yellow / red. Continue titration till yellow / red colour changes to orange indicating pH 4.4 – 4.5. Note the volume oType equation here.f H_2SO_4 required (B ml).
4. In case pink colour does not appear after addition of phenolphthalein continue as in 3 above.
5. Calculate total (T), Phenolphthalein (P) and Methyl orange (MO) alkalinity as follows and express as mg / l as $CaCO_3$.

OBSERVATIONS

S. No.	Volume of sample (ml)	Phenolphthalein indicator			Methyl orange indicator			Remarks
		Burette reading		Volume of NaOH run down (ml)	Burette reading		Volume of NaOH run down (ml)	
		Initial (ml)	Final (ml)		Initial (ml)	Final (ml)		

CALCULATION

Phenolphthalein alkalinity (P) mg/l as $CaCO_3$ = A x 1000/ml of sample taken

Methyl Orange alkalinity (M) mg/l as $CaCO_3$ = B x 1000/ml of sample taken

Total alkalinity(T) mg/l as $CaCO_3$ = (A+B) x 1000/ml of sample taken

Calculate the OH^-, CO_3^{--} and $HCO3^-$ forms from the values of P & T alkalinity as shown below.

Values of P&T	Alkalinity due to			
	OH^-	CO_3^{--}	HCO_3^{--}	pH Value
P = 0	0	0	T	8.2
P < ½ T	0	2P	T-2P	8.2 - 10.6
P = ½ T	0	2P	0	10.6
P > ½ T	2P - T	2(T – P)	0	10.6 –12.0
P = T	T	0	0	12.0

RESULT

Hydroxide alkalinity as $CaCO_3$ = mg/l

Carbonate alkalinity as $CaCO_3$ = mg/l

Bi-carbonate alkalinity as $CaCO_3$ = mg/l

IMPORTANCE

1. High alkaline water does not possess good taste.
2. Alkalinity causes precipitates and sludge's to be deposited in the pipes and heating cubes and also makes the metal brittle.
3. Alkalinity is a major quantity that must be considered in calculating the lime and soda ash requirement in water softening. The alkalinity of softened water is also a requirement for drinking water standards.
4. Chemicals used for coagulation of water and waste water reacts with water to give some acids. To destroy the acids released by the coagulant and for effective and complete coagulation, alkalinity must be present in excess.
5. Alkalinity measurements are made to evaluate the buffering capacity of wastewater and sludge.
6. Industrial wastes containing caustic alkalinity are prohibited from discharging into sewers.

Viva questions:

1. What is an alkalinity?
2. How it measured and what are its units?
3. What are the agents responsible for causing alkalinity?
4. What is the permissible limit for drinking water?
5. What are the disadvantages of Alkalinit

ACIDITY

EXPERIMENT-3 (b)

AIM

To determine the total acidity and mineral acidity of a given water sample.

APPARATUS

1. Burette
2. Pipette
3. Conical flask
4. Glazed tile

REAGENTS

1. Sodium hydroxide 0.02 N
2. Methyl Orange Indicator
3. Phenolphthalein Indicator

THEORY

Acidity of a liquid is its capacity to donate H^+ ions. The presence of acidity invariably indicates water pollution. Its presence indicates the discharge of acidic industrial effluents, acid mine drainages, pickling liquors and from humic acids.

Acidity is classified as mineral acidity (due to H_2SO_4 and HCl) and CO_2 acidity. The acidity present due to free CO_2 has no significance from public health point of view. Water containing mineral acidity is unacceptable.

PRINCIPLE

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

INTERFERENCE

Colour, Turbidity, Iron, Aluminum, and residual chlorine.

PROCEDURE

1. Take suitable volume of sample (20 ml) in 250 ml conical flask.
2. Add 2 drops of Methyl orange indicator. The sample turns pink/orange. Titrate with standard 0.02 N Sodium hydroxide solution till colour changes to yellow, characteristic pH is 4.3 – 4.4.
3. Note down the volume of NaOH required. (A ml)
4. Add 2 - 3 drops of phenolphthalein indicator and continue titration with NaOH till faint pink colour appears indicating pH 8.3
5. Note down the volume of additional NaOH required (B ml)

OBSERVATIONS

S. No.	Volume of sample (ml)	Methyl orange indicator			Phenolphthalein indicator			Remarks
		Burette reading		Volume of NaOH run down (ml)	Burette reading		Volume of NaOH run down (ml)	
		Initial (ml)	Final (ml)		Initial (ml)	Final (ml)		

CALCULATION

Mineral acidity as $CaCO_3$ in mg/l = A x 1000 / ml of sample taken

CO_2 acidity as $CaCO_3$ in mg/l = B x 1000 / ml of sample taken

RESULT

Mineral acidity of the given sample of water =

CO_2 acidity of the given sample of water =

Total acidity of the given sample of water = Mineral acidity + CO_2 acidity

Viva questions:

1. **What is acidity?**
2. **How it measured and what are its units?**
3. **What are the agents responsible for causing acidity?**
4. **What is the permissible limit for drinking water?**
5. **What are the disadvantages of acidity?**

CHLORIDES

EXPERIMENT-4

AIM

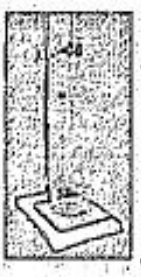
To estimate to the amount of chlorides present in the given sample of water.

APPARATUS

1. Pipette
2. Burette
3. Conical flask etc.

REAGENTS

1. Potassium chromate (K_2CrO_4) indicator
2. Silver nitrate ($AgNO_3$) of 0.0141N



THEORY

Chlorides occur widely in water and wastewater and are usually associated with sodium ion. Although chlorides are not harmful, concentrations beyond 250 mg/l impart a peculiar taste to water, rendering it unacceptable from aesthetic point of view for drinking purpose. Presence of chlorides above the usual background concentration in water sources is also used as an indicator of pollution by domestic sewage.

PRINCIPLE

Chlorides ion is determined by titration with standard $AgNO_3$ in which $AgCrO_4$ precipitates out. The end of titration is indicated by formation of red silver chromate from excess $AgNO_3$ and potassium chromate used as an indicator in neutral to slightly alkaline solution.

PROCEDURE

1. Adjust the pH of sample between 7.0 – 8.0.
2. Standardize $AgNO_3$ against standard NaCl solution.
3. Take 20ml well mixed sample and add 1.0ml of K_2CrO_4.
4. Titrate with standard $AgNO_3$ solution till $AgCrO_4$ starts precipitating.
5. For better accuracy titrate 20ml of distilled water in the same way to establish reagent blank.

OBSERVATIONS

S. No.	Volume of sample (ml)	Burette reading		Volume of $AgNO_3$ run down (ml)	Remarks
		Initial (ml)	Final (ml)		

CALCULATION

Chlorides (Cl^-) in mg/l = (A - B) x N x 35450/ml of sample

Where A = ml of $AgNO_3$ run down for the sample

B = ml of $AgNO_3$ run down for the blank.

N = Normality of $AgNO_3$ used.

RESULT

Chlorides present in the given sample = mg/l

Viva questions:

1. How it measured and what are its units?
2. What is the permissible limit for drinking water?
3. What are the disadvantages of Chlorides?
4. How irrigation is affected with excess chloride content?

Issues:

- Not harmful to human beings
- Regarding irrigation – most troublesome anion
- Acceptable limit - 250 mg/l

IRON

EXPERIMENT-5

AIM

To determine the Iron present in a given water sample.

PRINCIPLE

Iron in water under reduced state reacts with 1,10 Phenonthroline at PH 3.2-3.3 to form orange red complex. The intensity of colour produced is proportional to the concentration of iron. The intensity of colour is measured using colorimeter at 510 nm to determine the quantity of iron present in water.

APPARATUS

Spectrophotometer (of Colorimeter) (510 nm)

REAGENTS

(i) Concentrated Hydrochloric acid.
(ii) Hydroxylamine solution.
(iii) Ammonium acetate buffer solution.
(iv) Phenanthroline solution.
(v) Stock iron solution

PROCEDURE

1. Mix the sample and take 50 ml of the water sample. Add 2 ml con. HCL and 1 ml hydroxyl amine solution solution and boil the solution so as to reduce the volume to 20ml. Cool.
2. Add 10 ml ammonium acetate buffer and 2 ml orthophenonthroline solution and make up to 100 ml. Allow 10-15 minutes for maximum colour development. Run a distilled water blank along with the sample.
3. Take the absorbance readings by setting the distilled water blank to 100% transmission or zero absorb taking the readings of the colour developed in the sample at 510 nm.
4. Carry our a calibration graph by using the iron standards ranging from 0-100 µg Fe/100 ml by using 2 ml to 10 ml iron standards solution 1 ml =10 µg of Fe and following the procedure as mentioned above.
5. From the calibration curve determine the iron content.

CALCULATION

$$\text{Iron (mg/l)} = \frac{\mu g\ Fe}{ml\ of\ sample}$$

RESULTS

Sample volume (ml) =----------

Sample No. or Description	Colorimeter Reading (mg/l)	Iron Concentrarion

Issues:

- High iron causes brown or yellow staining of laundry, household fixtures.
- Metalic taste, offensive odour, poor tasting coffee
- Cause iron bacteria.
- Acceptable limit – 0.3 mg / l

Viva Questions:

1. How do presence of excess Iron in drinking can be perspective?
2. How it is measured?
3. What are the permissible limits in drinking water?
4. What are disadvantages of Iron

DISSOLVED OXYGEN (DO)

EXPERIMENT-6

AIM

To find the quantity of Dissolved oxygen present in the given sample.

APPARATUS

1. BOD bottles (capacity 300 ml)
2. Sampling device for collection of samples
3. Burette
4. Pipettes.

REAGENTS

1. Manganese Sulphate
2. Alkali iodide-azide reagent
3. Starch indicator
4. Standard sodium thio-sulphate (0.025 N)
5. Concentrated sulphuric acid (36 N)

PRINCIPLE

Oxygen present in sample oxidizes the divalent manganese to its higher valency, which precipitates as a brown hydrated oxide after addition of NaOH and KI. Upon acidification, manganese reverts to divalent state and liberates iodine from KI equivalent to D.O. content in the sample. The liberated iodine is titrated against $Na_2 S_2 O_3$ (0.25N), using starch as an indicator. If oxygen absent in sample, the $MnSO_4$ react with the alkali to from white precipitate $Mn(OH)_2$.

INTERFERENCE

PROCEDURE (WINKLER METHOD)

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

$$Mn^{++} + 2OH^{-} \longrightarrow Mn(OH)_2 \text{ (white)}$$
$$Mn^{++} + 2OH^{-} + {}^{1}/_{2}\, O_2 \longrightarrow MnO_2 \text{ (brown)} + H_2O.$$

5. After shaking and allowing sufficient time for all oxygen to react, the chemical precipitates are allowed to settle leaving clear liquid within the upper portion.
6. 2ml of concentrated sulphuric acid is added.
7. The bottle is restoppered and mixed by inverting until the suspension is completely dissolved and yellow colour is uniform throughout the bottle.

$$MnO_2 + 2I^{-} + 4H^{+} \text{-----> } Mn^{++} + I_2 + 2H_2O.$$

8. A volume of *203 ml is taken into the conical flask and titrated with 0.025 N sodium thiosulphate solution until yellow coloured iodine turns to a pale straw colour.
9. Since it is impossible to accurately titrate the sample to a colour less liquid, 1 to 2 ml of starch solution is added.
10. Continue titration to the first disappearance of the blue colour.

$$* \frac{200 \times 300}{(300 - 4)} = 203 \text{ ml}$$

OBSERVATIONS

S.No.	Volume of sample (ml)	Burette Readings		Volume of $Na_2S_2O_3$ run down (ml)	D.O in (mg/l)
		Initial (ml)	Final (ml)		

CALCULATIONS

1 ml of 0.025 N Na2S2O3 is equivalent to 0.2 mg of O_2, since the volume of the sample is 200 ml.

1 ml of sodium thiosulphate is equivalent to 0.2 x 1000 mg/l = 1 mg/l. O_2

RESULT

Dissolved oxygen concentration in the given sample = mg/l

Viva Questions:

1. What is importance of D.O?
2. Who is it measured and what are its units?
3. If D.O decreases what are the effects?
4. What is the permissible limit of D.O in drinking water?

NITRATE

EXPERIMENT-7

AIM

To determine the Nitrate present in a given water sample.

PRINCIPLE

Free chlorine interferes with Nitrate determination. Remove it by addition of one drop of sodium argentite solution for each 0.1 mg of chlorine.

APPARATUS

Spectrophotometer (or colorimeter) (410 nm).

REGENTS

(i) Brucinesulphanilinic acid solution
(ii) Sulphuric acid solution: sodium chloride solution
(iii) Stock nitrate solution

PROCEDURE.

- Take 10 ml sample in a tube, add 2 ml sodium chloride solution, mix thoroughly.
- Add 10 ml H_2So_4 solution mix, cool and add 0.5ml brucine sulphate reagent. Swirl the tube to get the solution mixed and place it in a water bath maintaining a temperature of about 95°C.After 20 minutes remove the tube and cool.
- Read the yellow color in a colorimeter at 410 nm.
- The calibration curve can be plotted using standards ranging from 0.1 to 1 mg/l by diluting 1-10 ml of the standard nitrate can be run for adjustment of colorimeter to 100% transmission or zero absorbance.

CALCULATION

$$\text{Nitrate N (mg/l)} = \frac{\mu g \text{ of Nitrate (N)}}{ml \text{ of Sample}} \times 100$$

Mg/l No 3 = mg of Nitrate (N) x 4.43

RESULTS

Sample description	Volume of Sample Sample (ml)	µg of Nitrate in the (mg/l)	Nitrate (mg/l)

REASONS:

- Increasing level of nitrate is due to Agricultural fertilizers, manure,animal dung, nitrogenous material ,sewage pollution

ISSUES:

- (blue baby diseases to infants)
- Maximum permissible limit 45 mg / l

Viva Questions:

1. How nitrates are contaminated in aquatic water?
2. How do you determine nitrates?
3. What is the difference between nitrite and nitrate?
4. What is permissible limit for Nitrates in drinking water
5. What are the disadvantages of nitrates?

OPTIMUM DOSES OF COAGULANT/ JAR TEST

EXPERIMENT-8

AIM

To find the optimum dose of coagulant required for treating the given turbid water sample.

APPARATUS

1. Jar test Apparatus.
2. pH meter.
3. One liter beakers - 6 Nos.
4. Graduated pipette.
5. Turbidity meter.

REAGENTS

Alum as coagulant solution.

PRINCIPLE

Very fine particles of size 1 to 500 nano meters of clay, micro-organisms, decomposing organic matter, phosphates, fluorides and certain toxicants remain suspended in water without settling and are called **COLLOIDS.**

Colloids: Hydrophobic (water hating)

Hydrophilic (water loving)

Hydrophobic colloids - Possessing no affinity for water are dependent on electrical charges for their stability in suspension. A coagulant destabilizes these colloids such that they contact agglomerate, form flocs and drop out of solution by sedimentation.

$$2Al^{+++} + \overset{---}{\underset{\text{Colloid}}{\quad}} \longrightarrow \underset{\text{Colloid}}{Al}$$

Flash mixing helps the coagulant intimately get mixed with colloids and then gentle mixing helps the particles to contact, and then to agglomerate.

Coagulation is dependent on pH, colour, turbidity, mineral matter, temperature, time of flocculation and degree of agitation.

The minimum concentration that effectively removes all the turbidity is the ideal dose of the coagulant.

PROCEDURE

1. Take the sample into each of the 6 beakers of one liter capacity.
2. Add varying doses of alum solution to different beakers simultaneously.
3. Switch on the motor and adjust the speed of the paddles to 100 rpm.
4. Allow flash mix (100 rpm) for about 1min to ensure complete dispersion of chemicals.
5. Reduce the speed of paddles to 40 rpm and continue mixing for 15 to 20 min.
6. Switch off the motor and allow the mixture for 30 min to settle the agglomerated particles.
7. Collect the supernatant without disturbing the sediment and find the turbidity.
8. Repeat the experiment with higher doses of alum if satisfactory results were not obtained.
9. Plot a graph between the % removal vs. dose of the coagulant and select the optimum dosage from the graph.
10. Also record the pH, colour, alkalinity and temperature.

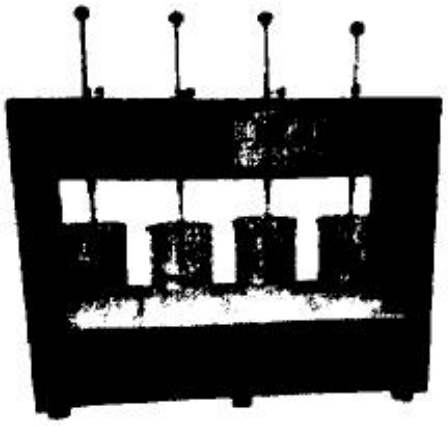
S.no	Alum doses	Turbidity

RESULTS

The optimum dosage of coagulant for the sample = mg/l

Viva Questions:

1. What is Jar test ?why it is done?
2. What is coagulant? And what is use?
3. Name some coagulant?
4. What is importance of coagulation process?



CHLORINE DEMAND/RESIDUAL CHLORINE

EXPERIMENT-9

AIM

To determine the residual chlorine in the given water sample.

APPARATUS

Chloroscope, Conical Flasks

REAGENTS

1. O-tolidine reagent
2. Sodium Arsenite solution
3. Copper sulphate solution
4. Potassium dichromate solution.

THEORY

Chlorine is almost universally employed in disinfecting water. It is cheap, reliable and presents no great difficulty in handling. Above all, it is capable of providing residual disinfecting effects for long periods, thus affording complete protection against future recontamination of water in the distribution system.

PRINCIPLE

When chlorine is added to water it reacts with water giving hypochlorous acid and hypochlorite ions which are efficient in killing bacteria.

$$Cl_2 + H_2O \longrightarrow HOCl + HCl$$

$$HOCl \longrightarrow H^+ + OCl^-$$

Chlorine in these forms in water is known as "Free available Chlorine". Chlorine being an active element is available in combination with ammonia or other nitrogenous compounds and is known as "Combined available Chlorine".

The Orthotolidine – arsenite test is used to find out the residual chlorine. The residual chlorine may be combined or free residual or both. When O-tolidine reagent is added to water, a yellow colour in

the sample indicates the presence of residual chlorine. The deeper the yellow colour the greater is the residual. These colours are compared with standard colours for exact determination of residual chlorine.

PROCEDURE

1. Use three conical flasks and designate them as A, B, C.
2. In conical flask A, add 1.0ml of O-tolidine reagent to 100ml sample. Thoroughly mix and add immediately 2ml of sodium arsenite solution.
3. After 2 minutes match the color with standard colour solutions.
4. This reading (FR) represents the free residual chlorine and any interfering substances.
5. In conical flask B, add 2ml of sodium arsenite solution to 100 ml of sample. Thoroughly mix and add immediately 1.0 ml of O-tolidine reagent.
6. After 2 minutes match the colour with standard solutions. This reading (B1) is for interfering substances.
7. Retain the solution and after 15 minutes again match the colour. This reading (B2) is for interfering substances.
8. In conical flask C, add 1.0 ml of O-tolidine reagent to 100 ml of the sample. Thoroughly mix and after 2 minutes match the colour.
9. This reading (TR) gives the total residual chlorine plus interfering substances.

CALCULATIONS

a) Free residual Chlorine as Cl, in mg/l = $FR - B_1$

b) Total residual Chlorine as Cl, in mg/l = $TR - B_2$

c) Combined residual Chlorine as Cl, in mg/l = $(TR - B_2) - (FR - B_1)$

RESULT

a) Free residual Chlorine as Cl = mg/l

b) Total residual Chlorine as Cl = mg/l

c) Combined residual Chlorine as Cl = mg/l

PRECAUTIONS

1. In this test it is required to control the temperature of water up to room temperature. Hence, in certain cases when the temperature of the sample is very cold, it may have to be slightly warmed, so as to bring it to room temperature. If this precaution is not observed the colour formation will be slow and uncertain.

2. When water tested is highly alkaline, a blue tinge is produced in the orthotolidine test. This may interfere with the measurement of yellow colour, to remove this tinge; the quantity of orthotolodine should be doubled.

RESIDUAL CHLORINE

- Chlorine added to water forms hypochlorite ions and hypochlorite acids
- Chlorine demand – Quantity required for killing micro-organisms and reacting with ammonia, organic compounds etc.
- To take care of post contamination
- Desirable – 0.2 mg / litre

Viva Questions:

1. What is the permissible limit of chlorine?
2. When chlorine becomes excess in water, what happens?
3. How do you measure excess of chlorine present in water?
4. What are the disadvantages of excess of chlorine?

METHOD #: 365.4	Pending Approval for NPDES, CWA (Issued 1974)
TITLE:	Phosphorous, Total (Colorimetric, Automated, Block Digester AA II)
ANALYTE:	CAS # P Phosphorus 7723-14-0
INSTRUMENTATION:	Autoanalyzer
STORET No.	00665

1.0 Scope and Application

1.1 This method covers the determination of total phosphorus in drinking water, surface water and domestic and industrial wastes. The applicable range of this method is 0.01 to 20 mg P/L.

2.0 Summary of Method

2.1 The sample is heated in the presence of sulfuric acid, K_2SO_4 and $HgSO_4$ for two and one half hours. The residue is cooled, diluted to 25 mL and placed on the AutoAnalyzer for phosphorus determination.

3.0 Sample Handling and Preservation

3.1 Sample containers may be of plastic material, such as a cubitainer, or of Pyrex glass.

3.2 If the analysis cannot be performed the day of collection, the sample should be preserved by the addition of 2 mL of conc. H_2SO_4 per liter and refrigeration at 4°C.

4.0 Apparatus

4.1 Block Digester BD-40

4.2 Technicon Method No. 327-74W for Phosphorus

5.0 Reagents

5.1 Mercuric sulfate: Dissolve 8 g red mercuric oxide (HgO) in 50 mL of 1:4 sulfuric acid (10 conc. H_2SO_4: 40 mL distilled water) and dilute to 100 mL with distilled water.

5.2 Digestion solution: (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of K_2SO_4 in 600 mL of distilled water and 200 mL of conc. H_2SO_4. Add 25 mL of mercuric sulfate solution (5.1) and dilute to 1 liter.

5.3 Sulfuric acid solution (0.72 N): Add 20 mL of conc. sulfuric acid to 800 of distilled water, mix and dilute to 1 liter.

5.4 Molybdate/antimony solution: Dissolve 8 g of ammonium molybdate and 0.2g of antimony potassium tartrate in about 800 mL of distilled water and dilute to

1967.
2. Gales, M.E. and Booth, R.L., "Evaluation of Organic Nitrogen Methods", EPA Office of Research and Monitoring, June, 1972.
3. Gales, M.E. and Booth, R.L., "Simultaneous and Automated Determination of Total Phosphorus and Total Kjeldahl Nitrogen", Methods Development and Quality Assurance Research Laboratory, May, 1974.
4. Technicon "Total Kjeldahl Nitrogen and Total Phosphorus BD-40 Digestion Procedure for Water", August, 1974.
5. Gales, M.E., and Booth, R.L., "Evaluation of the Technicon Block Digester System for the Measurement of Total Kjeldahl Nitrogen and Total Phosphorus", EPA-600 / 4-78-015, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

BIOCHEMICAL OXYGEN DEMAND (BOD)

EXPERIMENT 11

AIM

To determine Biochemical Oxygen Demand (BOD) exerted by the [illegible] sample

APPARATUS

1. BOD bottles (capacity 300 ml)
2. Sampling device for collection of samples
3. Burette
4. Pipettes

REAGENTS

1. Distilled water
2. Phosphate buffer solution
3. Magnesium sulphate solution
4. Calcium chloride solution
5. Sodium thio-sulphite solution.

PRINCIPLE

The BOD is an empirical biological test. This BOD test may be considered as wet oxidation procedure in which the living organisms serve as the medium for oxidation of the organic matter to carbon-di-oxide and water.

$$C_nH_aO_bN_c + [n + a/4 - b/2 - 3c/4]O_2 \xrightarrow{\text{Bacteria}} nCO_2 + [a/2 - 3c/2]H_2O + cNH_3$$

On the basis of the above relationship, it is possible to interpret BOD data in terms of organic matter as well as the amount of oxygen used during its oxidation.

INTERFERENCE

OBSERVATIONS

S.No.	Volume of Sample (ml)	Burette Readings		Volume of $Na_2S_2O_3$ run down (ml)	D.O in (mg/l)
		Initial (ml)	Final (ml)		

CALCULATIONS

Initial D.O. of ~~diluted~~ sample = D_o

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

Initial D.O. of distilled water (blank) = C_o

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

D.O. depletion of dilution water = $C_o - C_5$

D.O. depletion of the diluted sample = $D_o - D_5$

D.O. depletion of due to microbes = $D_o - D_5) - (C_o - C_5)$

BOD of the sample at 20^0 C = $\frac{[(D_o - D_5) \times \text{Vol. of the Bottle}] - (C_o - C_5)}{\text{ml of the sample}}$

RESULT

Bio-Chemical Oxygen Demand for the given sample = mg/l

Viva Questions:

1. What is the permissible limit of BOD?
2. When BOD becomes excess in water, what happens?
3. How do you measure excess of BOD present in water?
4. What are the disadvantages of excess of BOD?

BOD = $\frac{\text{Initial DO} - \text{final DO} \times \text{Vol. of bottle}}{\text{ml of the sample}}$

CHEMICAL OXYGEN DEMAND

EXPERIMENT-12

AIM

To determine the chemical oxygen demand of the given sample.

APPARATUS

1. Reflux apparatus
2. Hot plate / Heating mantle
3. Burette
4. Pipette
5. Measuring jars

REAGENTS

1. Standard potassium dichromate 0.25 N
2. Concentrated sulphuric acid reagent (H_2SO_4)
3. Silver sulphate (Ag_2SO_4)
4. Standard ferrous ammonium sulphate 0.1 N
5. Ferroin indicator
6. Mercuric sulphate

PRINCIPLE

Organic matter is oxidized to CO_2 and H_2O by boiling acid + dichromate solution.

$$8d\ H^+ + C_a\ H_b\ O_c + d\ Cr_2\ O_7^{2-} \xrightarrow{\text{Heat}} a\ CO_2 + eH_2O + 2c\ Cr^{3+}$$

Where $d = 2/3\ a + 1/6b - 1/3c$

$e = 1/2b + 4d$

The amount of dichromate, which has participated to get convert into Cr^{3+}, is identically equal to chemical oxygen demand of the sample and is determined by titration with ferrous ammonium sulphate solution using ferroin indicator

PROCEDURE

1. Take 20 ml of sample into a clean COD reflux flask.
2. Add 10ml of oxidizing agent ($K_2 Cr_2 O_7$) solutions are placed into flask together with glass beeds.
3. Add a pinch of mercuric sulphate silver nitrate and silver chloride.
4. Add 30 ml of concentrated H_2SO_4 and 3 or 4 glass beeds.
5. Attach the flask to the condenser and heat the flask on a hot plate at least for 2 hours to reflux the contents.
6. Cool the flask, detach from condenser unit and dilute the contents to about 140ml with distilled water.
7. Add 2 to 3 drops of ferroin indicator and titrate against ferrous ammonium sulphate solution till the reddish brown colour appears.
8. Repeat the above procedure for blank.

OBSERVATIONS

S. No.	Volume of the diluted sample (ml)	Burette reading		Volume of FAS run down (ml)	Remarks
		Initial (ml)	Final (ml)		

CALCULATION

The amount of COD for the given sample = $\frac{(P-Q) \times N \times 8000}{\text{ml of sample taken}}$ mg/l

P = Titrant volume for blank

Q = Titan volume of sample

N = Normality of FAS

RESULT

The amount of COD for the given sample = mg/l

Viva Questions:

1. What is the permissible limit of COD?
2. When COD becomes excess in water, what happens?
3. How do you measure excess of COD present in water?
4. What are the disadvantages of excess of COD?

TEST FOR COLIFORMS IN WATER

EXPERIMENT-13

AIM

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

PRINCIPLE

Coli form group comprises of all he aerobic, facultative and anaerobic gram-negative non-spore forming rod shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C. The standard test for this group may be carried out either by multiple tube fermentation technique or by membrane filter technique. The E.colitest by multiple tube fermentation technique consists of 3 phases – presumptive, confirmed and completed. Escherichia coli (E.coli)for the purpose of sanitary examination of water, is defined as a gram-negative, non-spore forming rod which is capable of fermenting lactose with the production of acid and gas at 35°C in less than48 hours, which produces in dole peptone water containing tryptophan, which is incapable of utilizing sodium citrate as its sole source of carbon, which is incapable of producing acetyl methyl carbine, and which gives a positive methyl red test. The results are expressed in terms of MPN (Most Probable Number), which is based on certain probability formulae. The estimate may give a value greater than the actual number of coli form present. The accuracy of any single test depends on the number of tubes fermented. This method helps in describing the sanitary quality of water. The safety of the water is generally judged from the knowledge of sanitary condition and mentioned by the number of samples yielding positive or negative results. If more than 95% should yield negative results, the safety is usually assured. The scheme of the MPN test is given as follows:

APPARATUS

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

REAGENTS

1. Lactose broth
2. Lauryl tryptose broth
3. Brilliant green lactose bile broth
4. Endo agar
5. Eosin methylene blue agar etc.

PROCEDURE

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

REAGENTS

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

PROCEDURE

PRESUMPTIVE TEST

1. Inoculate a series of fermentation tubes with appropriate graduated quantities (multiples and sub-multiples of 10) of the water to be tested. The concentration of nutritive ingredients in the mixture of the medium should conforms to the specifications. The partitions of the water sample used for inoculating lactose or laurel tryptose broth fermentation tubes will vary in size and number with the character of the water under examination. Usually, decimal multiples and sub-multiples of 1mL of the sample are selected. Inoculate 10 mL portion of each water sample provided into different one of the three large tubes containing 10 mL of lactose or lauryl tryptose broth which has been prepared with twice the normal concentration of constituent to allow for dilution. Inoculate 1.0 mL and 0.1 mL of water into small tubes (two sets of three each) of single strength lactose or laurel tryptose broth.
2. Incubate the inoculated fermentation tubes at 35±0.5°C. At the end of 24±2 hrs shake each tube gently and examine and if no gas is formed, repeat this test at the end of 48±3 hrs.

3. Record the presence or absence of gas formation at each examination of the tubes. Formation within48±3 hrs of gas in any amount in the inverted fermentation tubes constitutes a positive presumptive test. Active fermentation may be shown by the continued appearance of small bubbles of gas throughout the medium outside the inner vial in the fermentation tubes.

Presumptive test without confirmation should not be used routinely except in the analysis of heavily polluted water, sewage or other waste, which are not suitable for drinking purpose.

Calculation:-

Case (i)

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

Case (ii)

For other combinations and dilutions

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

= .....

Result:-

$$\frac{MPN}{100\ mL} = ...$$

Viva question:

1. Why coli form is carried out in water?
2. How do you determine? What are its units?
3. What is the permissible limit in water?
4. What are disadvantages if they excess in water?

SULPHATE

EXPERIMENT-14

1. Aim :- To Determine the Sulphates present in the given sample

PRINCIPLE 3

Sulphate if precipitated in hydrochloric acid medium with barium chloride to form barium sulphate. The absorbance of Barium sulphate suspension is measured in colorimeter.

APPARATUS 2.

(i) Spectrophotometer (or Colorimeter) for use at 420 nm
(ii) Stirrer

REAGENTS 4.

(i) Conditioning reagent
(ii) Barium chloride crystal
(iii) Standard sulphate solution

PROCEDURE 5.

- Measure 100 ml water sample or suitable portion made up to 100 ml.
- Add 5 ml condition reagent and a spoonful of $BaCL_2$ crystals. Stir for exactly 1 minute.
- Pour the solution in to the absorption cell and measure the turbidity in the colorimeter at 420 nm. Set the instrument to zero absorbance or 100% transmission using Distilled water.
- Take the absorbance reading of the sample.
- Prepare a calibration graph by using sulphate standards as described earlier. The standards can made from 0-40 mg/l sulphate range by taking 0-40 ml standard solution and making up to 100ml.

CALCULATION

$$\text{Sulphate } (So_4)\ (mg/l) = \frac{mg\ of\ (So^2)_4 x 1000}{ml\ of\ sample}$$

Corret for the colour and turbidity present in the original sample by running blank from which the $BaCl_2$ is withheld. In order to preserve the from deterioration, the sample may be kept at 4^0C before analysis.

OBSEVATONS FOR CALIBRATION GRAPH:

Sulphate standard (mg/l)	Colorimeter reading
[illegible] (mg/l)	[illegible]
0	0
10	0.161
20	0.234
30	0.291
40	0.398
50	0.536
60	0.642

RESULTS

SAMPLE VOLUME (ML)

Sample Description	Colorimeter reading	Sulphate Concentration (mg/l)

Comment:

TOTAL HARDNESS

EXPERIMENT-15

AIM

To determine the total hardness of a given sample of water

APPARATUS

1. Pipette
2. Burette
3. Conical flask etc.

REAGENTS

1. Buffer solution
2. Inhibitor
3. Erichrome black - T indicator.
4. Standard EDTA Solution 0.01M

THEORY

Originally water hardness was understood to be a measure of the capacity of water to precipitate soap. Soap is precipitated chiefly by calcium and magnesium ions present. Hardness is represented by the total concentrations of just the calcium and magnesium ions expressed as $CaCO_3$.

PRINCIPLE

In alkaline conditions EDTA reacts with Ca and Mg to form a soluble chelated complex. Ca and Mg ions develop wine red colour with Erichrome black-T under alkaline condition. When EDTA is added as a titrant the Ca and Mg divalent ions get complexed resulting a sharp change from wine red to blue, which indicates end point of the reaction. The pH for this titration has to be maintained at 10.0 ± 0.1

INTERFERENCE

Metal ions do interfere but can be overcome by addition of inhibitors

PROCEDURE