LAB MANUAL

ENVIRONMENTAL BIOTECHNOLOGY LAB

CODE: NBT-652

IMS ENGINEERING COLLEGE

A NAAC Accredited & ISO 9001:2008 Certified Institution

(Approved by AICTE and affiliated to APJ Abdul Kalam Technical University, Lucknow)

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EXPERIMENT: 1 **Date:**

OBJECTIVE: To determine the amount of dissolved oxygen in waste water by iodometric titration method.

THEORY

Adequate dissolved oxygen (DO) is necessary for the life of fish and other aquatic organisms. The DO concentration may also be associated with corrosivity of water, photosynthetic activity and septicity. DO level in natural and waste water is dependent on the physical, chemical and biochemical activities prevailing in the water body. The analysis of DO is key test in water pollution control activities and waste treatment process control.

The classical iodometric method of Winkler is a sensitive method for determining DO in water. The method essentially consists of addition of $MnSO_4$, KI and NaOH to the water sample when white $Mn(OH)_2$ is precipitated which is quickly oxidized by dissolved in water to brown $Mn(OH)_3$ and precipitated out.

$MnSO_4 \longrightarrow Mn^{2+} + SO_4^{2-}$

$KI \longrightarrow K^+ + I^-$

$NaOH \longrightarrow Na^+ + OH^-$

$Mn^{2+} + 2OH^- \longrightarrow Mn(OH)_2$: white precipitate

$4Mn(OH)_2 + O_2 + 2H_2O \longrightarrow 4Mn(OH)_3$ (s) : brown precipitate

The solution is acidified and Mn $(OH)_3$ oxidized KI, liberating equivalent quantity of free iodine which is then determined by titratng with standard sodium thiosulphate solution.

$H_2SO_4 \quad 2H^+ + SO_4^-$

$2Mn(OH)_3 + 2I^- + 6H^+ = 2Mn^{2+} + 6H_2O$

$I_2 + Na_2S_2O_3 = Na_2S_4O_6 + 2NaI$

In the determination of DO, there are various ions and compounds such as nitrite, ferrous iron, sulphate, thiosulphate, polythionate, free chloride, hypochloride, organic matters which are easily oxidized at the pH of alkaline iodide reagent cause interference in the DO analysis. To correct for these interferences various modification of the basic Winkler method have been proposed. The most common interference in biologically treated water in nitrite. The azide modification most effectively removes this interference.

REQUIREMENTS:

Reagents:

1. **Manganese sulphate solution:** Dissolve 480 gm of $MnSO_4.4H_2O$ or 400 gm of $MnSO_4.2H_2O$ or 300 gm of $MnSO_4.H_2O$ in distilled water and make up the volume to 1 liter. The manganese sulphate solution should liberate not more than a trace of iodine when added to an acidified KI solution.
2. **Alkali iodide-azide solution:** Dissolve 500 gm of NaOH and 150 gm of KI in distilled water and dilute to 1 liter. To this solution add 10 gm sodium azide (NaN_3) dissolved in 40 ml distilled water. This reagent should not give color with starch solution when diluted and acidified.

3. **Sodium thiosulphate solution (0.025 N):** Dissolve 6.3 gm of sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$) in boiled and cooled distilled water and make up the volume to 1 liter. Add 5 ml of chloroform as preservative and store in a dark bottle.
4. **Standard Potassium dichromate solution (0.025N):** Dissolve 1.22 gm potassium dichromate ($K_2Cr_2O_7$) in distilled water and make up the volume to 1 liter.
5. **Starch Indicator:** Prepare a paste of 1 gm of soluble starch with distilled water and add to this paste with constant stirring into 100 ml of boiled water and boil for one minute. Allow this solution to cool and add 3 gm of KI.
6. **Hydrochloric acid solution (1:1 by volume):** Add slowly 100 ml con. HCl in 100 ml distilled water in a beaker. Mix this solution thoroughly.
7. **Concentrated sulphuric acid (H_2SO_4)**
8. **Potassium iodide (KI)**
9. **Sodium hydrogen carbonate ($NaHCO_3$)**

REQUIREMENTS

Glassware: 300 ml BOD bottle, Beaker, Conical flask, Burette, Fixed volume pipette, Graduated pipette, Measuring cylinder, Watch glass, Wash bottle, Thermometer etc.

PROCEDURE

Standardization of sodium thiosulphate solution

Place 50 ml distilled water in a 500 ml conical flask; add 1 gm KI and 2 gm $NaHCO_3$ and shake until the salts dissolve. Add 12 ml of 1:1 HCl solution slowly whilst gently rotating the flask in order to mix the liquids. When evolution of CO_2 stops, run in 25 ml of standard $K_2Cr_2O_7$ solution, mix the solution well, cover the mouth with a watch glass, and allow standing for 5 minutes in order to complete the reaction. Titrate the liberated iodine with $Na_2S_2O_3$ solution, whilst constantly rotating the liquid so as to thoroughly mix the solution. When most of the iodine has been reacted as indicated by the solution acquiring a faint brown color, add 2 ml starch solution and continue the titration until the blue color of the starch-iodine complex disappears.

$$Normality\ of\ \ Na_2S_2O_3\ solution = \frac{V_1 N_1}{V_2}$$

V_1 = volume of standard $K_2Cr_2O_7$ solution taken
N_1 = normality of $K_2Cr_2O_7$ solution
V_2 = volume of $Na_2S_2O_3$ solution required for titration

Dissolved Oxygen determination

Collect the water sample in 300 ml BOD bottle, taking precaution to avoid entrainment or dissolution of atmospheric oxygen while filling the bottle. Add 2 ml manganese sulphate solution followed by 2 ml alkali iodide azide to the sample collected. The reagent should be added with a pipette, the tip of which should be well below the liquid surface in the bottle. Stopper the bottle with care to exclude air bubbles; mix the contents by inverting the bottle several times. When the precipitate settles, leaving a clear supernatant above the manganese hydroxide floc, shake again and allow settling. When settling has produced at least 100 ml clear supernatant, carefully remove the stopper and immediately add 2 ml of con. H_2SO_4 by allowing the acid run down the neck of the bottle, re-stopper and mix by gently inverting the bottle until dissolution of precipitate is complete. Take 100 ml of the original solution in a 500 ml conical flask after correction for the loss of sample by displacement with the reagents has been made. Thus, when a total of 4 ml (2 ml each) of manganese sulphate and alkali

iodide azide reagents are added to 300 ml BOD bottle, the original volume of sample is reduced to 296 ml due to overflow of sample. Hence actual volume to be taken for titration which corresponds to 100 ml original sample is

$$100\ [300 / (300-4)] = 101.5\ \text{ml}$$

and titrate with standard sodium thiosulphate solution to a pale yellow color. Add 2 ml of starch solution and continue titration to the first disappearance of the blue color.

RESULTS

Determine the concentration of dissolved oxygen in mg per ml in the water sample.

DO (mg/ml) = 80 x V x N

V = volume of sodium thiosulphate solution
N = normality of sodium thiosulphate solution

Report the standard DO concentration of water at the sample temperature.

PRECAUTIONS

1. After the addition of reagents in water sample taken inside BOD bottle, the bubble check is done and its removal is done if formed. Failing which the oxygen of air present inside the bubble will also take part in the reaction and this will lead to high value of DO.
2. The reagents should be added inside the bottle with the help of pipette to avoid more air contact.
3. As far as possible, the sample should not be allowed to come in contact with air.

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EXPERIMENT: 2 **Date:**

OBJECTIVE: To determine the Biochemical Oxygen Demand (BOD) of a given waste water sample

THEORY

The oxygen demand of sewage, sewage plant effluents, polluted waters or industrial wastes is exerted by three classes of materials:

1. Carbonaceous organic material usable as a source of food by aerobic organisms.
2. Oxidizable nitrogen derived from nitrite, ammonia and organic nitrogen compounds which serve as food for specific bacteria (e.g. Nitrosomonas and Nitrobacter).
3. Certain chemical reducing compounds (ferrous iron, sulfate and sulfide) which will react with molecularly dissolved oxygen.

In raw and settled and domestic sewage, most and for practical purposes, all of the oxygen demand is due to the first class of materials and is determined by biochemical oxygen demand, (BOD) test.

The BOD test is based upon determination of dissolved oxygen prior to and following a 5 days incubation period of the sample at 20^0C. A known volume of sample of sewage is diluted to with a known volume of dilution water (water containing nutrients for a bacterial growth). The dissolved oxygen of this solution is predetermined (D_1). Now, this solution is kept for incubation for a period of 5 days and DO of this solution is determined (D_2).

$$BOD = D_1 - D_2 \; x \; \frac{volume\ of\ \ sample\ after\ dilution}{volume\ of\ \ sample\ before\ dilution}$$

D_1 = DO of diluted water sample after its preparation (immediately)
D_2 = DO of diluted water sample after incubation of 5 days at 20 ^{0}C

REQUIREMENTS:

Glassware

300 ml BOD bottle, Beaker, Conical flask, Burette, Fixed volume pipette, Graduated pipette, Measuring cylinder, Watch glass, Wash bottle, Thermometer etc.

Reagents

1. **Phosphate buffer solution:** Dissolve 8.5 gm $KH_2PO_4.7H_2O$ and 21.75 gm Na_2HPO_4 and 1.7 gm NH_4Cl in boiled out distilled water and make the volume to 1 litre.
2. **Magnesium sulfate solution:** Dissolve 22.5 gm of $MgSO_4.7H_2O$ in distilled water and dilute to 1 litre.
3. **Ferric chloride solution:** Dissolve 0.25 gm of $FeCl_3.6H_2O$ in distilled water and dilute to 1 litre.
4. **Calcium chloride solution:** Dissolve 27.5 gm $CaCl_2$ in distilled water and dilute to 1 litre.
5. **Dilution water:** In 1 litre of de-mineralized (distilled, tap or natural) water add 1 ml phosphate buffer solution, 1 ml of magnesium sulfate solution, 1 ml of ferric chloride solution and 1 ml of calcium chloride solution.

PROCEDURE

Take 1 litre of dilution water in a flat bottom flask. Purge air through the solution for about 30 minutes to saturation the dilution water with oxygen. The dilution water is seeded with the addition of 20 ml of domestic sewage which has been stored at 20 ^{0}C for about 24 hours.
Take 3 BOD bottles. Add 10 ml (or as per instructions) of waste water sample in two bottles. Fill the 3 bottles with seeded dilution water. The bottle without sample will act as blank. Measure Do of one bottle containing sample. Allow the other two bottles for incubation for 5 days at 20 ^{0}C. After the incubation period measure the DO of the sample bottle as well as blank bottle.

RESULTS

BOD of the given water sample in mg/ml =

$$BOD = D_1 - D_2 \; x \; \frac{volume\ of\ \ sample\ after\ dilution}{volume\ of\ \ sample\ before\ dilution}$$

D_1 = DO of diluted water sample after its preparation (immediately)
D_2 = DO of diluted water sample after incubation of 5 days at 20 ^{0}C

PRECAUTIONS

1. BOD bottle should be cleaned with a detergent and washed with water before its use.
2. The BOD of sample should be determined at the earliest as during storage between collection and storage BOD decreases.
3. While determining DO by iodometric method and applying azide modifications, the presence of bubble inside the bottle should be checked and immediately remove.
4. BOD bottle with samples, during incubation, should not come in the contact of light otherwise photosynthetic production of DO occurs.

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EXPERIMENT: 3 **Date:**

OBJECTIVE: To determine Chemical Oxygen Demand (COD) in a given waste water sample.

THEORY

Chemical oxygen demand is a measure of any kind of oxidisable impurities present in the sewage. COD is a measure of both the biologically oxidisable and biologically inert organic matter present in the sewage sample. It is an important and quickly measured parameter for stream and industrial waste water analysis and water treatment plants.

Most type of organic matter is oxidized by a boiling mixture of chromic and sulfuric acids. A measured quantity of sample is refluxed in strongly acid solution with a known excess of potassium dichromate. After digestion, the excess dichromate remaining unreacted is titrated with ferrous ammonium sulfate solution using Ferrion as an indicator. The potassium dichromate consumed is proportional to the amount of oxidizable impurities in the given water sample. Keep ratios of reagent weights, volumes and strengths constant when sample volumes other than 50 ml are used. The standard 2 hour reflux time may be reduced if it has been shown that a shorter period yields the same results.

REQUIREMENTS

Reagents

1. **Standard Potassium dichromate solution (0.25N):** Dissolve 12.259 gm potassium dichromate ($K_2Cr_2O_7$) previously dried at 103^0C in distilled water and make up the volume to 1 liter.
2. **Sulfuric acid-silver sulfate reagent:** Add 4.4 gm silver sulfate in 1 liter concentrated sulfuric acid and keep over night for complete dissolution.
3. **Ferroin Indicator:** Dissolve 1.485 gm 1,10-phenanthroline monohydrate and 695 mg $FeSO_4.7H_2O$ in distilled water and dilute to 100 ml.
4. **Standard Ferrous Ammonium Sulfate solution (0.25 N):** Dissolve 98 gm of FAS in distilled water. Add 20 ml con. Sulfuric acid, cool and dilute to 1000 ml.
5. **Mercuric Sulfate.**

REQUIREMENTS:

Apparatus: Reflux apparatus consisting of 500 ml flask and condensers.

Glassware: Beaker, Conical flask, Burette, Fixed volume pipette, Graduated pipette, Measuring cylinder, Wash bottle etc.

PROCEDURE

Standardization of Ferrous Ammonium Sulfate solution

Dilute 25 ml standard $K_2Cr_2O_7$ to about 100 ml. Add 30 ml con. H_2SO_4 and cool. Titrate with FAS titrant using 2 to 3 drops ferrion indicator.

$$Normality\ of\ \ FAS\ solution = \frac{V_1 N_1}{V_2}$$

V_1 = volume of standard $K_2Cr_2O_7$ solution taken

N_1 = normality of $K_2Cr_2O_7$ solution
V_2 = volume of FAS solution required for titration

Chemical Oxygen Demand determination

1. Place 0.4 gm mercuric sulfate in reflux flask.
2. Take 50 ml of waste water sample by pipette in reflux flask and add dew glass beads in the flask.
3. Add 25 ml standard potassium dichromate solution by pipette.
4. Add slowly 75 ml of sulfuric acid-silver sulfate solution mixing thoroughly along with swirling of the flask during the addition.
5. Mix well. If the solution turns green, throw the solution and take smaller amount of sample diluted to 40 ml.
6. Connect the flask to condenser and reflux the contents of the flask on heating unit for 1.5 to 2 hours.
7. Cool the flask and add distilled water so that the final volume of the flask is about to 300 ml.
8. Titrate the excess $K_2Cr_2O_7$ with 0.25 N FAS using 5 drops of ferrion indicator. Titrate continuously as at the end point the solution turns red sharply.
9. Prepare a reagent blank along with the sample taking 50 ml distilled water in place of sample.

RESULTS

$$COD\ in\ \ mg/l = \frac{(A-B)\ x\ N\ x\ 1000}{ml\ of\ \ sample\ used}$$

A = ml of FAS required for blank
B = ml of FAS required for sample
N = normality of FAS

PRECAUTIONS

1. Adding of sulphuric acid reagent to reflux should be done slowly and by shaking the flask. As it is an exothermic reaction the flask should be cooled during mixing.
2. Smaller volume of sample should be taken in the flask, if it has high COD.
3. End point of the titration should be carefully observed.

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EXPERIMENT: 4 **Date:**

OBJECTIVE: To determine total, temporary (carbonate), permanent (non-carbonate) hardness of water sample by complexometric titration (EDTA) method.

THEORY

Calcium and magnesium salts as well as salts of polyvalent metals such as iron, almonium manganese, strontium and zinc, when present in water, from insoluble precipitate with soap and the water is called "hard water". Hardness of water is, therefore the sum of concentrations of all those soap precipitating ions. In general, calcium and magnesium salts are the principle cause of water hardness and therefore the hardness is generally expressed in terms of calcium carbonate, $CaCO_3$, equivalence of all salts and reported as mg of $CaCO_3$ per litre of water sample.

Hardness due to carbonates and bicarbonates is called "carbonate hardness" or "temporary hardness". Carbonate hardness of water can be removed easily by boiling when carbonates of calcium and magnesium are precipitated out. Hardness due to other salts is called "non-carbonate" or "permanent hardness" and cannot be removed by boiling.

Since most of the hardness is due to calcium and magnesium salts, these can be determined easily by complexometric titration method using EDTA solution.

Ethylenediaminetetraacitic acid and its sodium salts (EDTA) from a chelated soluble complex when added to a solution of certain metal ions such as calcium and magnesium. If a small quantity of dye such as Solochrome black (Eriochrome black T) is added to an aqueous solution containing calcium and magnesium ions at a pH of 10.0, the solution will become wine red. If EDTA is then added as a titrant, the calcium and magnesium will be complexed. After sufficient EDTA has been added to complex all the calcium and magnesium, the solution will turn from wine red to blue. This is the end point of the titration.

Titration should be conducted at or near room temperature for satisfactory color change of the indicator. At a pH value of 10.0 calcium carbonate precipitates out slowly and hence titration would give erroneous result if taken long time to perform. A time limit of 5 minutes for the overall procedure minimizes the error to an acceptable limit.

Some metal ions as well as suspended or colloidal organic matter, if present beyond a certain limit, will interfere with the titration and therefore addition of some inhibitors.

REQUIREMENTS

Glassware: Beaker, Conical flask, Burette, Fixed volume pipette, Graduated pipette, Measuring cylinder, Wash bottle etc.

Reagents

1. **Buffer solution:** Dissolve 1.179 gm AR di-sodium salt of EDTA and 0.78 gm of $MgSO_4.7H_2O$ in 50 ml distilled water. Add this solution to 16.9 gm NH_4Cl and 143 ml of concentrated NH_4OH with mixing and dilute to 250 ml with distilled water.
2. **EDTA solution (0.1 M):** Dissolve 3.723 gm di-sodium salt of EDTA in distilled water and make up the volume to 1 liter and store in a polythylene bottle.
3. **Standard Calcium chloride solution:** Weigh 1 gm of AR calcium carbonate in a 500 ml conical flask and add drop wise 1:1 HCl until all the carbonate are dissolved. Add

about 200 ml distilled water and boil for a few minutes to expel CO_2. Cool, add a few drops of methyl orange indicator and adjust to intermediate orange color by adding 3N NH_4OH or 1: HCl, as required. Transfer quantitatively to 1 litre volumetric flask fill to the mark with distilled water. This standard solution is equivalent to 1 mg $CaCO_3$ per 1ml.

4. **Eriochrome Black-T indicator solution:** Dissolve 0.5 gm of Eriochrome Black-T in 100 ml alcohol. Indicator solution should be used within about 6 weeks from the date of its preparation.
5. **Hydrochloric acid solution (1:1 by volume):** Add slowly 100 ml con. HCl in 100 ml distilled water in a beaker. Mix this solution thoroughly.
6. **Ammonium chloride solution (3N):** Add 21 ml concentrated NH_4OH solution in 79 ml distilled water and mix thoroughly.

PROCEDURE

Standard the EDTA solution as follows

Pipette out 25 ml of standard calcium solution in a 250 ml conical flask. Add 25 ml distilled water, 2 ml buffer solution and 5 drops of Erichrome Black-T indicator solution. Titrate with EDTA solution until the color changes from wine red to blue. No tinge of redish hue should remain at equivalence point. Titrate slowly near the end point. Calculate the $CaCO_3$ equivalent of EDTA solution from the relation.

$$1ml\ EDTA\ solutiion = \frac{S\ x V_1}{V_2}\ mg\ CaCO_3$$

V_1 = volume of standard calcium solution taken, ml
V_2 = volume of EDTA required, ml
S = $CaCO_3$/ml of standard solution

Take 50 ml of water sample in a 250 ml conical flask and add 2 ml of buffer solution and 5 drops of Erichrome Black-T indicator solution. Titrate with standard EDTA solution until the wine red color changes to blue. Repeat this with 2 more aliquots of 50 ml each. Let this volume be VT. This titration measures total hardness.

Take 250 ml of sample in a 500 ml beaker and boil gently for 20-30 minutes. Cool and filter it directly in a 500 ml conical flask. Do not wash the filter paper. Add 10 ml of buffer solution and 5 drops of Erichrome Black-T indicator solution. Titrate with standard EDTA. Let this volume be VP. This titration measures the permanent hardness.

RESULTS

Calculate the total hardness from VT, permanent hardness from VP and temporary hardness by difference.

$$Hardness(EDTA)\ as\ mg/1CaCO_3 = \frac{A\ x\ B\ x 1000}{ml\ of\ \ sample}$$

A = ml titration for sample
B = mg $CaCO_3$ equivalent to 1.00 ml EDTA titrant

PRECAUTIONS

1. All the solution should be freshly prepared.
2. The same amount of the indicator must be added each time.
3. The reaction mixture should be briskly shaken during the titration.
4. The end point should be observed correctly.
5. pH 10 should be maintained during the titration.

[Signature of Student] **[Signature of Instructor]**

EXPERIMENT: 5 **Date:**

OBJECTIVE: To determine total solids (TS), total dissolved solids (TDS) and total suspended solids (TSS) in a given water sample.

THEORY

A well-mixed sample is evaporated in a weighed dish and dried to constant weight in an oven at 103 to105 ^{0}C. The increase in weight over that of the empty dish represents the total solids. The results may not represent the weight of actual dissolved and suspended solids in waste water samples.

A well-mixed sample is filtered through a standard glass-fiber filter, and the filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 180 ^{0}C. The increase in dish weight represents the total dissolved solids. This procedure may be used for drying at other temperatures.

A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105 ^{0}C. The increase in weight of the filter represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, it may be necessary to increase the diameter of the filter or decrease the sample volume. To obtain an estimate to total suspended solids, calculate the difference between total dissolved solids and total solids.

REQUIREMENTS

Apparatus: Hot air oven at 104 ^{0}C, Heater etc.

Glassware: Dissicator, Measuring Cylinder, Funnel, Beaker 100 ml, Filter paper etc.

PROCEDURE

Total Solids

1. Weight the given beaker accurately and record the reading.
2. Shake the sample so that the sample is homogenous. Measure 50 ml sample with measuring cylinder and transfer it to the pre-weighed beaker.
3. Put the beaker on the heater covered with wire gauge.
4. Evaporate the sample till only a small amount of water remains in the beaker.
5. Transfer the beaker in the hot air oven at 104 ^{0}C. Allow it to remain there for half an hour. Place the beaker in the dessicator and weigh till you get a constant weight.
6. The difference between the two weights of beaker gives the amount of total solids (TDS + TSS) present in the sample.

Total Suspended Solids

1. Weight the given filter paper accurately.
2. Place it in the funnel and filter 50 ml of well shaken sample using suction pump
3. Place the filter in the hot air oven for half an hour so that the filter paper and constants are completely dry.
4. Place the dried filter paper in the dessicator and weight till you get constant reading.
5. The difference between the two weights of filter paper will give the amount of total suspended solids (TSS) present in the sample.
6. Similarly the difference in weights of TS and TSS will give the amount of TDS present in the sample.
7. Record the amount of TDS and TSS in mg/L.

RESULTS

$$Total\ solids\ mg/l = \frac{(A-B)x1000}{volume\ of\ sample,\ ml}$$

A = weight of dried residue + beaker, mg
B = weight of beaker, mg

$$Total\ suspended\ solids\ \ mg/l = \frac{(A-B)x1000}{volume\ of\ sample,\ mg/l}$$

A = weight of filter + dried residue, mg
B = weight of filter, mg

$$Total\ dissolved\ solids = Total\ solids - Total\ suspended\ solids$$

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EXPERIMENT: 6 **Date:**

OBJECTIVE: To determine the total alkalinity of the given sample of water using standard acid solution

THEORY

The alkalinity of water is the capacity of that water to accept protons. The alkalinity of water is due to the presence of hydroxide ion (OH^-), carbonate ion (CO_3^{-2}) and bicarbonate ion (HCO_3^-) present in the given sample of water. These can be estimated separately by titration against standard acid, using phenolphthalein and methyl orange indicators. Phenolphthalein indicator enables the measurement of that alkalinity fraction contributed by the hydroxide and half of the carbonate. Phenolphthalein alkalinity responding in the pH range 8-10. Indicators responding in the pH range 4-5 are used to measure the alkalinity contributed by hydroxide, carbonate and bicarbonate. This type of alkalinity is called the total alkalinity or methyl orange alkalinity. The chemical reaction involved can be shown by the equation given below.

$$OH^- + H^+ \rightarrow H_2O \quad (1)$$

$$CO_3^{2-} + H^+ \rightarrow HCO_3^- \quad (2)$$

$$HCO_3^- + H^+ \rightarrow H_2O + CO_2 \quad (3)$$

The titration of water sample against a standard acid up to phenolphthalein end point shows the completion of reactions 1 and 2 only. The amount of acid used thus correspond hydroxide plus half of the carbonate present.

The Titration of the water sample against standard acid to methyl orange end point shows the completion of reaction 1, 2 and 3.

REQUIREMENTS:

Glassware: Beaker, Conical flask, Burette, Fixed volume pipette, Graduated pipette, Measuring cylinder, Wash bottle etc.

Reagents

1. Standard Hydrochloric Acid (0.02 N)
2. Phenolphthalein Indicator
3. Methyl Orange Indicator

PROCEDURE

Phenolphthalein alkalinity

Take 100 ml of sample in a 250 ml conical flask. Add 1-2 drops of phenolphthalein indicator. If the sample turns pink, titrate this sample against standard acid solution (0.02 N HCl) till the pink color disappears. Note the volume of acid used (P ml).

Total alkalinity or methyl orange alkalinity

Take 100 ml of sample in a 250 ml conical flask. Add 1-2 drops of methyl orange indicator. It will give light yellow color. Titrate this sample against standard acid solution (0.02 N HCl) till the light yellow color changes to red. Note the volume of acid used (T ml).

RESULTS

$$Phenolphthlaein\ alkalinity\ as\ mg/l\ CaCO_3 = \frac{P\ x\ N\ x\ 50{,}000}{ml\ sample}$$

$$Total\ alkalinity\ as\ mg/l\ CaCO_3 = \frac{T\ x\ N\ x\ 50{,}000}{ml\ sample}$$

N = normality of acid solution

There are five combinations for P and T.

1. If P = 0, total alkalinity due to bicarbonates
2. If P = less than ½ T
   Alkalinity due to carbonates = 2P
   Alkalinity due to the bicarbonates = T - 2P
3. If P = more than ½ T
   Alkalinity due to carbonates = 2 (T - P)
   Alkalinity due to the hydroxide = 2P - T
4. If P = ½ T, total alkalinity due to carbonates
5. If P = T, alkalinity due to hydroxide only

PRECAUTIONS

1. The glass should be perfectly clean before the start of the experiment.
2. Since phenolphthalein and methyl orange indicators are used in determination of alkalinity the end point of the titration should be observed carefully.

[Signature of Student] **[Signature of Instructor]**

EXPERIMENT: 7 **Date:**

OBJECTIVE: To determine the acidity of a waste water sample

THEORY

The acidity of water is the capacity of that water to donate protons. This includes the un-ionized portions of weakly ionizing acids such as carbonic acid and tannic acid, as well as hydrolyzing salts. Mineral acids contribute to acidity when the sample has a low pH value. The acidity is significant because acids contribute to the corrosiveness of water.

Equilibrium between carbonate, bicarbonate and carbon dioxide exists in many natural waters used for portable purposes. The carbonate and bicarbonate can be estimated by titrating the alkalinity with standard acid to the bicarbonate equivalence point of pH 8.3 and then to the carbonic and equivalence point in the pH range of 4 to 5. Acid pollutants entering a water supply in sufficient quantity will disturb the carbonate-bicarbonate-carbon dioxide equilibrium. The extent of this disturbance may be estimated by titrating with standard alkali to the endpoints of pH 4.5 and 8.3.

For mineral acids, the titration is carried to a pH of about 4.5 by using methyl orange indicator, giving a color change from red to yellow. The acidity thus determined is called methyl orange acidity. Total acidity or phenolphthalein acidity is determined by carrying the titration to phenolphthalein end point 8.3. The results are expressed as parts of equivalent $CaCO_3$ per million parts of water.

REQUIREMENTS

Glassware: Beaker, Conical flask, Burette, Fixed volume pipette, Graduated pipette, Measuring cylinder, Wash bottle etc.

Reagents

1. Sodium Hydroxide solution (0.02N)
2. Phenolphthalein Indicator
3. Methyl Orange Indicator

PROCEDURE

Methyl Orange acidity

Take 100 ml of sample in a 250 ml conical flask. Add 1-2 drops of methyl orange indicator. If it gives an orangish red color it means mineral acidity is available. Titrate with sodium hydroxide solution (0.02 N) till the yellow end point. Note the volume of sodium hydroxide used.

Total acidity or phenolphthalein acidity

Take 100 ml of sample in a 250 ml conical flask. Add 1-2 drops of phenolphthalein indicator. If it does not give any color, titrate with sodium hydroxide (0.02 N) to a pink end point. Note the volume of sodium hydroxide used. If phenolphthalein gives a pink color on addition in the sample, acidity is not available.

RESULTS

Methyl orange acidity or mineral acidity, mg/l =
Phenolphthalein acidity or CO_2 acidity, mg/l =

$$Acidity\ as\ mg/1CaCO_3 = \frac{A\,x\,N\,x\,50{,}000}{ml\ sample}$$

A = ml titration for sample
N = normality of NaOH

PRECAUTIONS

1. The cleaned apparatus should be used for the titration.
2. To avoid loss of CO_2, the titration should be carried out quickly and vigorous shaking should be avoided.

[Signature of Student] **[Signature of Instructor]**

EXPERIMENT: 8 **Date:**

OBJECTIVE: To determine the concentration of hexavalent chromium in the given water sample

THEORY

The carcinogenic potential of hexavalent chromium is a good reason to protect a potable water supply against its intrusion. The hexavalent chromium concentration of drinking water has been reported to vary between 0.0003 and 0.04 mg/l with a mean of 0.0032 mg/l. Chromium salts are used extensively in industrial processes and may enter a water supply through the discharge of waters. Chromium compounds are frequently added to cooling water for corrosion control. Chromium may exist in water supplies in both the hexavalent and the trivalent state, although the trivalent from rarely occurs in potable water supplies.

Hexavalent chromium reacts with diphenylcarbazide to produce a reddish-purple color in slightly acid solutions.

In the color development step the following substances may cause interference. Mercury, both mercurous and mercuric, gives a blue or blue-purple color, but the reaction is not very sensitive at the acidity employed. Iron in concentrations greater than 1 mg/l interferes by producing a yellow color with the reagent. Vanadium interferences in the same manner but more strongly. The color produced with vanadium fades fairly rapidly and is negligible 10 min after the addition of the diphenylcarbazide.

REQUIREMENTS

Apparatus: Spectrophotometer

Glassware: Beaker, Volumetric flask, fixed volume pipette, Graduated pipette, measuring cylinder, Wash bottle etc.

Reagents

1. **Stock Chromium solution:** Dissolve 11.3 mg of $K_2Cr_2O_7$ in 250 ml of distilled water.
2. **Diphenyl Carbazide solution:** Dissolve 250 mg of 1, 5,-Diphenyl Carbazide in 50 ml of acetone.
3. **Sulfuric acid solution (6N):** Add slowly 10 ml of concentrated sulfuric acid in 50 ml of distilled water.

PROCEDURE

1. Prepare standard solution of chromium, taking 25 ml of stock chromium solution with the help of a pipette and dilute to 250 ml in a volumetric flask with distilled water.
2. Take 0, 5, 10, 15, 20, 25 and 30 ml of the standard solution in different 100 ml volumetric flask using pipette and 10 ml (or as per instructions of the instructor) unknown sample each in three 100 ml volumetric flask.
3. In each sample add 2 ml of diphenyl carbazide reagent followed by 2 ml of 6N H_2SO_4.
4. Make up the volume to 100 ml by distilled water of each sample.
5. Measure the absorbance of standard solutions and unknown samples at 540 nm on a spectrophotometer with respect to blank sample as reference.

RESULTS

Plot absorbance vs concentration of Cr^{6+} and prepare a calibration curve. Check whether Lambert-Beers Law holds for the concentration range of your experiment.

Calculate the Cr^{6+} concentration of the unknown sample from the calibration curve and absorbance readings.

CALCULATION

$$294\ gm\ K_2Cr_2O_7\ contains = 104\ gm\ of\ Cr^{6+}$$

$$11.3x10^{-3}\ gm\ K_2Cr_2O_7\ contains = \frac{104}{294}x11.3x10^{-3}\ gm\ of\ Cr^{6+} = A$$

$$Con.\ of\ Cr^{6+}\ in\ the\ stock\ solution = \frac{A}{250}\ gm/ml$$

$$Con.\ of\ Cr^{6+}\ in\ the\ standard\ solution = \frac{25\,x\,\frac{A}{250}}{250}\ gm/ml = B$$

$$Con.\ of\ Cr^{6+}\ in\ 5ml\ sample = \frac{B\,x\,5}{100}\ gm/ml$$

Similarly calculate the con. of Cr^{6+} in other standard sample for preparing the calibration curve.

[Signature of Student] **[Signature of Instructor]**

EXPERIMENT: 8 **Date:**

OBJECTIVE: To determine the amount of nitrate in waste water by Spectrophotometric method

THEORY

Nitrite, an intermediate stage in the nitrogen cycle may occur in water as a result of the biological decomposition of proteinaceous materials. Nitrite may also be produced in water treatment plants or in the distribution system through the action of bacteria or other organisms on ammonia nitrogen fed at elevated temperature in the combined residual chlorination of water. Nitrite can be likewise enters a water supply system through its use as a corrosion inhibitor in industrial process water.

The determination of nitrite ion in water is important in assessing the degree of pollution. The efficiency of water purification process can be judged by the amount of nitrite ion in the water.

Nitrite ion can be determined in water by utilizing the reaction of this ion with amines (diazotization). The compound 4-aminobenzenesulphonic acid is diazotized according to the reaction:

$$HSO_3^- \text{-}NH_2 + NO_2 + 2H^+ = HSO_3^- \text{-}N^+{=}N + 2H_2O$$

The diazonium salt is then coupled with 1-naphthylamine at pH 2.0 to 2.5 (with sodium acetate) to form the reddish-purple coloured azo dye:

$$HSO_3^- \text{-}N^+{=}N \longrightarrow HSO_3^- \text{-}N{=}N\text{-} \quad \text{-}NH_2 + H^+$$

The solution is made slightly basic with sodium acetate to make this reaction complete.

REQUIREMENTS

Apparatus; Spectrophotometer

Glassware: Beaker, Volumetric flask, fixed volume pipette, Graduated pipette, measuring cylinder, Wash bottle etc.

Reagents

1. **Sulfanilic acid solution:** Dissolve about 0.8 g of sulfanilic acid (4-amino-benznesulfonic acid) in 28 ml of glacial acetic acid and dilute the solution to about 100 ml with distilled water.
2. **Naphthyamine solution:** Dissolve about 0.5 g of 1-naphthylamine in 28 ml of glacial acetic acid and dilute the solution to about 100 ml with distilled water.
3. **Sodium acetate solution:** Dissolve about 14 g of sodium acetate trihydrate in distilled water and dilute to about 50 ml.
4. **Standard nitrite solution:** Weigh accurately 0.247 g of AR grade sodium nitrite, dissolve the salt in distilled water, and dilute the solution to 500 ml in a volumetric flask. Pipette 5 ml of this solution into another 500 ml volumetric flask and dilute the solution to the mark. This solution now contains 0.0010mg of nitrite nitrogen per ml.

PROCEDURE

Into nine 100 ml volumetric flasks, pipette 0, 1, 2, 3, 4, 5, 6, 7, and 10 ml portions of the standard nitrite solution. Adjust the volume in each flask to about 50 ml with distilled water. Add to each flask 1 ml of the sulfanilic acid solution and allow the solution to stand for 5 minutes. Then add to each flask 1 ml of the 1-napthylamine solution and 1 ml of the sodium acetate solution. Finally, dilute each solution to the mark. These solutions constitute the standard nitrite solutions for preparing the calibration curve.

Into four 100 ml volumetric flasks, pipette 5, 5, 10, 10, ml (or as per instruction of the instructor) water sample and treat it in the same way as done in the case of standard nitrite sample.

Measure the absorbance of each standard solutions and the unknown at 520 nm.

RESULT

Plot the absorbance vs. the concentration of the standards and note whether Lambert Beer's law is obeyed.

From the absorbance of the unknown solution, calculate the number of milligrams of nitrogen per litre (ppm) in the original solution.

CALCULATION

$$Concentration\ of\ \ nitrite\ in\ sample\ 1 = \frac{1x10^{-6}\ g/ml\ x1ml}{100\ ml}$$

Similarly calculate the concentration of nitrite in other standard sample for preparing the calibration curve.

[Signature of Student] **[Signature of Instructor]**

EXPERIMENT: 10 **Date:**

OBJECTIVE: To determine the amount of sulphate ion in the sample by Nephelometric method.

THEORY

Sodium and magnesium sulphates exert a cathartic action. Therefore the recommended sulphate concentration in potable water is limited to 250 mg per litre. Waste water particularly industrial waste water may contain high percentage of sulphate and when it is discharged in river or open land the concentration of sulphate of the river water or ground water may increase beyond the acceptable limit.

Sulphate in water may be determined by turbidimetric method. Sulphate ions are precipitated in a hydrochloric acid medium with barium chloride in such manner as to form barium sulphate crystals of uniform size. There are no ions other than sulphate in normal water that will form insoluble compounds with barium under strongly acid conditions. The amount of barium sulphate in the suspension is determined by measuring the turbidity of the solution with nephelometer and using a calibration curve.

Colour or suspended matter in large amounts will interfere with this method. Some suspended matter may be removed by filtration. If both are small in comparison with the suphate ion concentration, interference is corrected for using blank run. Silica in excess of 500 mg/1 will interfere, and, in water containing large quantities of organic materials, it may not be possible to precipitate barium sulphate satisfactorily.

The turbidity of a dilute barium sulphate suspension is difficult to reproduce; it is therefore essential to adhere rigidly to the experimental procedure detailed below. The velocity of precipitaion, as well as the concentration of the reactants, must be controlled by adding pure solid barium chloride of definite grain size. The rate of solution of barium chloride controls the velocity of reaction. Sodium chloride and hydrochloric acid are added the before the precipitation in order to inhibit the growth of microcrystals of barium sulphate; the optimum pH is maintained and minimizes the effect of variable amount of other electrolytes present in the sample upon the size of the suspended barium sulphate particles. A glycerol-ethanol solution helps to stabilize the turbidity. The reaction vessel is shaken gently in order to obtain a uniform particle size. Each vessel must be shaken at the same rate and the same number of times. The unknown must be treated exactly like the standard solution. The interval between the time of precipitation and measurement must be kept constant.

REQUIREMENTS

Reagents

1. **Conditioning reagent:** Mix 50 ml glycerol with a solution containing 30 ml conc. HCl, 300 ml distilled water, 100 ml 95% ethyl alcohol and 75 gm sodium chloride.
2. **Barium chloride:** Use crystals of A.R. barium chloride that pass through a 20- mesh sieve and are retained on 30 mesh screen.
3. **Stanadard sulphate solution:** Weigh out accurately 0.1814 gm dry AR potassium sulphate in a 1000 ml volumetric flask and dissolve in distilled water and make up to the mark. Calculate the concentration of sulphate ion in the solution.

PROCEDURE

Pipette out 0.0, 10.0, 20.0, 30.0, 40.0, and 50.0 ml of the standard potassium sulphate solution and three 50 ml of unknown solutions (or as per instruction of the instructor) in to separate 100 ml volumetric flasks. To each flask add 5.0 ml conditioning reagent and mix thoroughly and dilute to 100 ml with distilled water.

Add 0.3 g of barium chloride crystals in each flask and shake for 1 minute by inverting the flask once per second. All the barium chloride should dissolved. Allow the flask to stand for 2-3 minute and measure the turbidity of the suspension in a nephelometer. Use the blank sample to adjust zero of the instrument and most concentrated sample to adjust instrument reading to 100 by means of sensitivity control. Recheck again instrument 0 and 100 reading and adjust if necessary. Measure the turbidity of the remaining samples.

RESULTS

Plot nephelometer reading vs sulphate concentration of the standard samples.
Determine the sulphate concentration of the unknown from the graph.

[Signature of Student] **[Signature of Instructor]**

EXPERIMENT: 11 **Date:**

OBJECTIVE: To conduct jar tests on a sample of natural surface water in order to estimate an optimum dosage of ferric chloride for removal of suspended matter

THEORY

Mining operations use **flocculation** to settle out sludge from mine drainage and from tailings pond effluent. The sludge comes from metals in solutions that come from the mine and the concentrator. In the flotation process, after the desired metals such as copper are skimmed off, the remaining slurry is sent to the tailings pond. After initial settling, the solids in the slurry are sent to a Floc tank where chemical reagents are added which causes the remaining solid material to settle out. The clarified water is then either recycled back to the concentrator or undergoes further treatment and is released into a natural waterway. For much of its operating life, the Sullivan Mine at Kimberly, B.C., and release untreated mine waters into the local drainage system causing extensive pollution. In 1979 the mine constructed a mine water treatment system that uses flocculation to clarify and treat water which can be discharged directly into the St. Mary River. Sludge is sent directly to a designated waste site area. Now the previously polluted areas have rebounded back to healthier ecosystem due to the water treatment facility at the mine.

Flocculation refers to the successful collisions that occur when the hydraulic shear forces in the rapid mixing of flocculation basins drive the destabilized particles toward each other. Agglomerates of a few colloids then quickly bridge together to form microflocs, which in turn gather into visible flock masses. These flock masses then precipitate out of solution. Ultimately, the flocculent helps separate the sludge from the water.

Flocculation is an important unit process used in wastewater treatment. Flocculation involves a less intense mixing to promote the aggregation of destabilized particles into larger flocks that can be removed subsequently by sedimentation and/or filtration. Prior to the filtering of fermentation broth some additives are added to collect the biomass in greater flocks. The filtering process is much faster when the flocks are greater.

REQUIREMENTS

Reagents

1. 1 L Ferric chloride solution ($FeC1_3 * 6H_2O$), 10 mg Fe_2O_3 /ml
2. 1 L 0.1 M Na_2CO_3
3. Six-place laboratory stirrer
4. Six 1 L beakers
5. Turbidometer
6. PH meter
7. Approximately 10 L Buckeye River Water
8. Indicators, chemical solutions, and glassware for alkalinity measurements

PROCEDURE

1. Analyze the collected natural surface water for pH, turbidity, and alkalinity. Record both the water temperature at time of test and ambient air temperature.
2. Calculate the amount of alkalinity required to react with the maximum dosage of ferric chloride (50mg/L as Fe_2CO_3 so that the resulting alkalinity will be at least 0.5 meq/L 25 mg/L as $CaCO_3$ so that the resulting alkalinity will be at least 0.5 meq/L 25 mg/l as $CaCO_3$ equivalent) if the reaction is complete. Measure the pH.

3. Place the flocculated slurry into the filter paper-lined funnel in the second beaker. The sludge should have settled by this time to the bottom of the beaker. The filtered water should be clear of any solid material and look as clear, if not more clear than tap water.
4. Remove the sludge using a spoon or garden hand tool, and put it into a planting pot.
5. Carefully mix peat moss into the sludge to help hold moisture and loosen the soil. Use approximately 1 part moss for every 3 parts sludge.
6. Plant 4-6 marigold seeds, or others, according to package instructions.
7. Fill a second pot with potting soil and plant 4-6 marigold seeds in the soil.
8. Place the pots either on a windowsill or in greenhouse, if one is available at the school.
9. Check the posts daily and record in your journal when the seeds germinate and sprout, and record their growth (i.e. height). Note the difference between the plants growing in the tailings and potting soil. Use the filtered water to water the plants.

ANALYSIS:

1. Prepare tables/graphs that facilitate comparisons of coagulant dosages with alkalinity, pH, turbidity, and other changes observed. Plot turbidity vs. coagulant dosage as part of the analysis.
2. Select the optimum dosage on the basis of supernatant clarity and settle ability of flock with secondary considerations to cost and sludge production.
3. Estimate the volume of sludge that might be produced by treating water.

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