Water laboratory booklet

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Water and microbiology of water

1-1- Introduction

The water laboratory is one of the chemical engineering laboratories. The main purpose of this laboratory is to conduct chemical, environmental and microbiological tests in the field of water and wastewater in a practical way. These tests are the main ones in the field of water and sewage. The procedures described in these standards are intended for the examination of waters of a wide range of quality, including water suitable for domestic or industrial supplies, surface water, ground water, cooling or circulating water, boiler water, boiler feed water, treated and untreated municipal or industrial wastewater, and saline water. The unity of the fields of water supply, receiving water quality, and wastewater treatment and disposal is recognized by presenting methods of analysis for each constituent in a single section for all types of waters. An effort has been made to present methods that apply generally. Where alternative methods are necessary for samples of different composition, the basis for selecting the most appropriate method is presented as clearly as possible. However, samples with extreme concentrations or otherwise unusual compositions or characteristics may present difficulties that preclude the direct use of these methods. Hence, some modification of a procedure may be necessary in specific instances. Whenever a procedure is modified, the analyst should state plainly the nature of modification in the report of results. Certain procedures are intended for use with sludges and sediments. Here again, the effort has been to present methods of the widest possible application,

but when chemical sludges or slurries or other samples of highly unusual composition are encountered, the methods of this manual may require modification or may be inappropriate.

1-2- Safety in Laboratory

1-2-1- Personal Safety

Acquaintance of students with the general experiments, equipment and devices tested by the laboratory expert, which must be approved by the laboratory manager.

Extinguishing the fire: Fire extinguishing devices are placed in a special place inside or outside the laboratory (depending on the conditions and the possibility of danger) (identify the location of the fire extinguishing capsule upon entering each laboratory).

Shoes: The shoes should be comfortable and have rubber soles and cover the whole foot. Whenever there is a possibility of spilling materials, disposable covers that are resistant to the penetration of liquids should be worn. Cloth shoes should not be used because they absorb chemicals and infectious liquids. It is recommended to use shoes made of materials impermeable to liquids, such as leather or synthetic materials.

Use of jewelry and ornaments: You should not use jewelry and ornaments except for the wedding ring (in cases that do not conflict with the principles of safety and health). Because they may get stuck in equipment or hang inside contaminated materials or dissolve in chemicals.

Eye washing: In case of eye and face contamination with chemicals, the best way is to wash quickly with a large amount of water for 15 minutes.

Hand contact: Avoid touching the face, eyes, ears, nose, etc. while working in the laboratory. Prohibited eating and drinking: In all laboratories, eating food and drinking beverages and other actions that cause hand-to-mouth contact are prohibited. Many of the substances inside the laboratories are very toxic and carcinogenic

Use of gloves: Gloves should always be available in different sizes and made of appropriate and quality materials in all technical departments. Latex, nitrile or vinyl gloves provide adequate protection. Gloves made of thin latex or vinyl do not provide adequate protection against punctures by sharp objects. Gloves should be available in wrist, elbow and shoulder sizes.

It is mandatory for all students to use a lab coat while working in the lab and avoid placing clothes and personal items on the table or platforms of laboratories

Before performing each experiment, note down the required chemicals and glassware and make the necessary coordination with the laboratory expert.

After finishing the work, hand over your used equipment to the laboratory expert after washing.

Every student is obliged to do her best in maintaining the laboratory devices, which are almost impossible to replace in the current conditions.

Working with acids, organic solvents and other volatile and dangerous substances is allowed only under the hood and with full observance of safety principles.

1-2-2- Necessary precautions in using laboratory equipment

After removing the tools and using them, clean each one and put it in its own place.

Always try to take out a little bit of the chemical when weighing the materials, do not return a lot of it to its original container (unless with the permission of the laboratory manager).

You should not use any tool to remove the powders, but you should use steel spatulas (spatulas) that are made for this purpose, and after each use, wash it with distilled water and water, and after drying, use it again. used them.

When using glassware, you must make sure that they are healthy.

When filling containers with valve, including burettes, it is necessary to make sure that their valve is closed.

Before connecting the electrical devices to the electric current, you have to pay enough attention to the type of current and the amount of voltage determined for the device

To perform accurate tests, place sensitive electrical devices in a suitable place, so that they do not communicate with each other and do not move.

1-2-3- Necessary precautions in using chemicals

Due to the fact that most chemicals are harmful to humans, direct contact of chemicals with the skin, mouth and other parts should be avoided. In case of contamination of hands and face, it should be cleaned immediately with soap and water or another suitable solvent

The use of foam is mandatory when drawing dangerous solutions by pipette, especially in the case of solutions that contain toxic vapors.

If chemicals enter your mouth, you should not swallow it, but immediately remove it from your mouth and rinse your mouth with plenty of water. If the
said substance was acid, dilute the acid by drinking plenty of water and never vomit. If the eaten substance was alkaline, use anti-alkaline agents such as vinegar, lemon juice, and yogurt.

If you eat heavy metals, use milk or egg whites that absorb the above metals. In the case of arsenic and mercury, force yourself to vomit by drinking warm water and salt and using mechanical stimulation.

In preparing mixed solutions of acid and base, be careful to add the acid drop by drop and slowly to the water.

It is recommended to use plastic glasses to protect the eyes from possible splashing of substances into the eye.

Total Dissolved Solids Dried at 180°C

2-1- Purpose of Experiment

The purpose is the determination of Total residue and Total dissolved (filterble) solid of water at 180°C

2-2- Outcome of Experiment

During this experiment, students learn that dissolved solids in water can be organic or inorganic. Understanding the TDS value of water and the amount of dissolved solids present in the water gives an accurate picture of water quality. In this experiment we will talk about total soluble solids in water, how to measure them and how to reduce total dissolved solids in water.

2-3- Application of concepts in industry

TDS measurement is common in various fields such as hydrology, water treatment industry, aquaculture and food industry. Measuring the TDS of water is very important in various industries. The World Health Organization recommends a TDS level of less than 300 mg L^{-1} for drinking water and 50-150 is excellent for drinking. However, this value can vary depending on local regulations and standards. TDS mechanism is established by the Government to collect tax at source. It helps prevent tax evasion to an extent, as the deductors file all the TDS deduction details in quarterly TDS returns.

2-4- Theory of Experiment

Total dissolved solids are the total amount of solids dissolved in the water, including soluble hydrogen carbonate ions, chloride salts, sulphates, calcium, magnesium, sodium, potassium, volatile solids and non-volatile solids. To determine the total dissolved solids in a solution 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°C. The increase in dish weight represents the total dissolved solids. This procedure may be used for drying at other temperatures. The results may not agree with the theoretical value for solids calculated from chemical analysis of sample. Approximate methods for correlating chemical analysis with dissolved solids are available. The filtrate from the total suspended solids determination may be used for determination of total dissolved solids.

2-5- Apparatus

a. Evaporating dishes: Dishes of 100-mL capacity made of one of the following materials:
b. Porcelain, 90-mm diam.
c. Platinum—Generally satisfactory for all purposes. High-silica glass.
d. Steam bath.
e. Desiccator, provided with a desiccant containing a color indicator of moisture concentration or an instrumental indicator.
f. Drying oven, for operation at 103 to 105°C.

g. Analytical balance, capable of weighing to 0.1 mg.

h. Magnetic stirrer with TFE stirring bar.

i. Wide-bore pipets.

j. Graduated cylinder.

k. Low-form beaker.

figure1. Steam bath

figure 2. Deccicator

Figure3. Drying Oven

Figure4. Analytical balance

figure 5. Magnetic Stirrer

2-6- Procedure

a. Preparation of glass-fiber filter disk: If pre-prepared glass fiber filter disks are used, eliminate this step. Insert disk with wrinkled side up into filtration apparatus. Apply vacuum and wash disk with three successive 20-mL volumes of reagent-grade water. Continue suction to remove all traces of water. Discard washings.

b. Preparation of evaporating dish: If volatile solids are to be measured, ignite cleaned evaporating dish at 550°C for 1 h in a muffle furnace. If only total dissolved solids are to be measured, heat clean dish to 180 ±

c. 2°C for 1 h in an oven. Store in desiccator until needed. Weigh immediately before use.

d. Selection of filter and sample sizes: Choose sample volume to yield between 2.5 and 200 mg dried residue. If more than 10 min are required to complete filtration, increase filter size or decrease sample volume.

e. Sample analysis: Stir sample with a magnetic stirrer and pipet a measured volume onto a glass-fiber filter with applied vacuum. Wash with three successive 10-mL volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 min after filtration is complete. Transfer total filtrate (with washings) to a weighed evaporating dish and evaporate to dryness on a steam bath or in a drying oven. If necessary, add successive portions to the same dish after

evaporation. Dry evaporated sample for at least 1 h in an oven at 180 ± 2°C, cool in a desiccator to balance temperature, and weigh. Repeat drying cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of previous weight or 0.5 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight. If volatile solids are to be determined, follow procedure to Fixed and Volatile Solids Ignited at 550°C.

2-7- Calculation:

$$\text{mg total dissolved solids}/L = \frac{(A - B)\times 1000}{\text{sample volume mL}}$$

where:

A = weight of dried residue + dish, mg, and

B = weight of dish, mg.

2-8- question:

I. Define the following terms:

Residue

Total Residue

Total Filterable Residue

Total Nonfilterble Residue

Total volatile & Fixed SResidue at 550°

II. Explain the importance of TDS measurment

Conductivity of Solution

3-1- Purpose of Experiment

The aim of this experiment is to determine and consider the conductivity of water and the importance of conductivity measurement.

3-2- Outcome of Experiment

During this experiment, students learn that the electrical conductivity in water environments, such as irrigation water or soil water, indicates the amount of dissolved mineral salts and is an measure of the amount of salts and salinity in soil and water, so that the quality and classification of water and soil Salinity is determined by EC. Electrical Conductivity measurement indirectly determines the amount of dissolved solutes in water and is determined by field and laboratory methods and the unit measure it mS c^{-1} or mmoh cm^{-1}.

3-3- Application of concepts in industry

Measuring and controlling the concentration and dilution of some acids and inorganic minerals, fuels and solutions in chemical, petrochemical and refining industries is necessary. In food and processing industries, measuring the concentration and dilution of materials used in CIP processes for washing juice lines and juice concentrates, soft drinks, malt beer and brewer's yeast, dairy products, sugar and sugar cane, paste, sauce is perfomrmed. Surveillance of feedwater purity, control of drinking water and process water quality, estimation of the total number of ions in a

solution or direct measurement of components in process solutions can all be performed using conductivity measurements.

3-4- Theory of Experiment

Electrical conductivity, k, is a measure of the ability of a solution to carry an electric current. This ability depends on the presence of ions; on their total concentration, mobility, and valence; and on the temperature of measurement. Solutions of most inorganic compounds are relatively conductive. Conversely, molecules of organi compounds that do not dissociate in aqueous solution conduct a current very poorly.

3-5- Apparatus and Reagent

a. Conductivity instruments (Condutivitymeter)

Use an instrument capable of measuring conductivity with an error not exceeding 1% or 1 µmho/cm, whichever is greater.

b. Conductivity water

c. Standard potassium chloride solution, KCl, 0.0100M: Dissolve 745.6 mg anhydrous KCl in conductivity water and dilute to 1000 mL in a class A volumetric flask at 25°C and store in a CO2-free atmosphere. This is the standard reference solution, which at 25°C has a conductivity of 1412 µmhos/cm.

3-6- Procedure

Rinse conductivity cell with at least three portions of 0.01M KCl solution. Adjust temperature of a fourth portion to 25.0 ± 0.1°C. If a conductivity meter displays resistance, R, ohms, measure resistance of this portion and note temperature. Compute cell constant, C:

$$C,\ cm^{-1} = (0.001412)\ (R_{KCL})\ [1 + 0.0191(t - 25)]$$

where:

R_{KCL} = measured resistance, ohms, and

t = observed temperature, °C.

Conductivity meters often indicate conductivity directly. Commercial probes commonly contain a temperature sensor. With such instruments, rinse probe three times with 0.0100M KCl, as above. Adjust temperature compensation dial to 0.0191 C−1. With probe in standard KCl solution, adjust meter to read 1412 µmho/cm. This procedure automatically adjusts cell constant internal to the meter. b. Conductivity measurement: Thoroughly rinse cell with one or more portions of sample. Adjust temperature of a final portion to about 25°C. Measure sample resistance or conductivity and note temperature to ±0.1°C.

3-7- Calculation:

The conductivity in µmho/cm is given by the equation:

$$G = 1{,}000{,}000C/R\ [(1 + 0.0200(T - 25)]$$

Where R= Resistance of the sample ohm

C= Cell constant

T = Temperature °C

3-8- Questions

I. Explain how to obtain TDS from condutivity measurement.

II. Explain the application of conductivity.

Alkalinity

4-1- Purpose of Experiment

The object of this Experiment is measuring the alkalinity of water. The alkalinity of a water sample is its quantitative capacity to neutralize a strong acid to a designated pH .

4-2- Outcome of Experiment

During this experiment, students learn that alkalinity is one of the influential factors in water sedimentation and its corrosion factor. In steam boilers, the alkalinity of the water protects the walls of the boilers from corrosion. However, the alkalinity should be controlled. Its high amount causes transfer of solids with steam. Cracks may also appear on the walls. Alkalinity itself is also related to pH level. The higher the simple alkalinity, the higher the amount of hydroxides. It shows carbonates. As a result, the alkalinity of the water increases. As mentioned, the main source of alkalinity is carbonate compounds, especially limestone. The solubility of calcite and dolomite in water is low. However, in contact with the atmosphere and due to the presence of CO2, their solubility increases greatly. A change in the amount of CO2 also changes the amount of bicarbonate. However, by increasing it beyond the balance, it leads to an increase in the solubility of calcium carbonate and an increase in water alkalinity. On the contrary, reduction of CO2 leads to reduction of alkalinity.

4-3- Application of concepts in industry

Water operation in the cooling tower means a set of measures and measures necessary to prevent damage to the cooling tower by sediments. Water treatment in the cooling tower is usually used to control item alkalinity, which determines the size of the calcium carbonate particles. Alkalinity of wastewater in the presence of a large amount of carbon dioxide, usually in the range of 30-50%, in the anaerobic treatment gas, usually the alkalinity values in the range of 2000-4000 mg L^{-1} based on calcium carbonate are needed to maintain the pH in a neutral state or close to The state is neutral. The required amount of alkalinity is rarely available in the incoming wastewater; But it is possible that in some cases it can be produced by the decomposition of protein and amino acids (such as meat packaging wastewater), the need to purchase chemicals for pH control can have an important effect on the economic efficiency of anaerobic treatment. The relationship between pH and alkalinity of wastewater is controlled by bicarbonate.

4-4- Theory of Experiment

Alkalinity of a water is its acid-neutralizing capacity. It is the sum of all the Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid. Alkalinity thus depends on the end-point pH used. For methods of determining inflection points from titration curves and the rationale for titrating to fixed pH end points. For samples of low alkalinity (less than 20 mg $CaCO_3$/L) use an extrapolation technique based on the near proportionality of concentration of hydrogen ions to excess of titrant beyond the equivalence point. The

amount of standard acid required to reduce pH exactly 0.30 pH unit is measured carefully. Because this change in pH corresponds to an exact doubling of the hydrogen ion concentration, a simple extrapolation can be made to the equivalence point.

End points: When alkalinity is due entirely to carbonate or bicarbonate content, the pH at the equivalence point of the titration is determined by the concentration of carbon dioxide (CO_2) at that stage. CO_2 concentration depends, in turn, on the total carbonate species originally present and any losses that may have occurred during titration. ‘‘Phenolphthalein alkalinity’’is the term traditionally used for the quantity measured by titration to pH 8.3 irrespective of the colored indicator, if any, used in the determination. Phenolphthalein or metacresol purple may be used for alkalinity titration to pH 8.3. Bromcresol green or a mixed bromcresol green-methyl red indicator may be used for pH 4.5.

Interferences: Soaps, oily matter, suspended solids, or precipitates may coat the glass electrode and cause a sluggish response. Allow additional time between titrant additions to let electrode come to equilibrium or clean the electrodes occasionally. Do not filter, dilute, concentrate, or alter sample.

4-5- Apparatus and Reagent

a. Electrometric titrator or pH meter

b. Magnetic stirrer

c. Glasswares (Flasks, Pipet, Burets, Polyolefin bottle)

d. Sodium carbonate solution 0.05 N

e. Standard sulfuric acid or hyd rochloric acid 0.1 N
f. Standard sulfuric acid or hyd rochloric acid 0.02 N
g. Mixed bromcresol green-methyl red indicator solution
h. Methyl orange Soloution
i. Phenolphthalein solution
j. Sodium thiosulfate 0.1 N
k. Balance

Figure1.pH meter   Figure2. Magnetic stirrer   Figure3. Balance

4-6- Procedure

Potentiometric Titration to PH 3.7 or 8.3

Rinse the electrode & Titration vessels with distilled water. Select sample size (50 ml) and discharge into an erlenmayer flask. Measure the PH of the sample. Add standard acid in increments, Mix Thoroughly, Avoiding splashing. When PH-value 8.3 (phenolphthalein end point) is approached record the vol ume of acid. Continue until pHval ue approach 3.7 (methyl orange end point) then stop titration. Record the volume of acid at PH=3.7For low alkalinities (less than 20 mg/l it) Stop the titration at a pH in the

range 4.3 to 4.7 and record the volume of acid and exact pH very carefully Add additional titrant to lower the pH of the soloution exactly 0.3 pH unit and again record the volume.

4-7- Calculation:

Potentiometric titration to end-point pH

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times N \times 50000}{\text{ml sample}}$$

where:

A = mL standard acid used and

N = normality of standard acid

Or

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times t \times 1000}{\text{mL sample}}$$

A = mL standard acid used and

t = titer of standard acid, mg CaCO3 /mL.

Report pH of end point used as follows: ‘‘The alkalinity to pH _______ = _______ mg CaCO3 /L’’ and indicate clearly if this pH corresponds to an inflection point of the titration curve

$$\text{Total alkalinity, mg CaCO3 / L} = \frac{(2B - C) \times N \times 50000}{\text{mL sample}}$$

where:

B= ml titrant to first recorded pH,

C = total ml titrant to reach pH 0.3 unit lower, and

N = normality of acid

4-8- Questions

I) What are the methods of alkalizing? Describe them and write their advantages and disadvantages.

II) What is the method of keeping the pH of hot pool water and hot tub water constant based on alkalinity?

Hardness Measurement

5-1- Purpose of Experiment

The purpose of this experiment is the standardizantion of the "EDTA solution and determination of the hardness and calcium of water samples.

5-2- Outcome of Experiment

During this experiment, students learn that water hardness determines the quality of water. Among these, water-soluble salts are one of the most important factors. All the salts that are soluble in water represent impurities in water. Students also learn that hardness is classified into two categories: carbonate (temporary) hardness and non-carbonate (permanent) hardness. So, two groups of anions can be placed against the cation of the hardness agent (calcium or magnesium). If the anion that opposes these factors is carbonate or bicarbonate, we will have temporary hardness. But if other anions are placed against them, we will have permanent difficulty. Anions such as nitrate, sulfate and chloride create non-carbonate hardness.

5-3- Application of concepts in industry:

Water becomes more concentrated due to contact with minerals and water-soluble solutes such as calcium, magnesium, aluminum, iron, and manganese, and its degree of hardness increases, which is why it is called "hard water".

Calcium and magnesium sulfate leads to the formation of deposits in water heater pipes, power generating facilities and household water pipes.

Calcium and magnesium chlorides soluble in water cause corrosion of boiler walls and water pipes.

Waters with a very hard degree cause saltiness and a bad taste due to the presence of sodium chloride more than 300 mg.

The presence of many salts in water has a negative effect on the color quality in dyeing and textile industries

Excessive hardness of water will lead to digestive diseases and the formation of kidney and bladder stones

Detergent power of soapy substances and detergents is greatly reduced in hard water

Therefore, water hardness is used to reduce or eliminate water hardness.

It is necessary to use hardeners in powrehouses, some residential areas, hospitals and many production and industrial units. The hardness remover is also used to remove the hardness of drinking water, remove the hardness of the water entering the boiler and steam boiler, and remove the hardness of the water in the cooling towers.

Water hardness has advantages and disadvantages. Its advantages include the following:

Softened water prolongs the useful life of equipment and devices such as pipes and household appliances.

Softened water makes soaps and detergents work more effectively.

Softened water is better for some people's skin and hair.

Hardener saves money and energy.

5-4- Theory of Experiment

The hardness of water was understood to be a measure of the capacity of the water for precipitating soap. Soap is precipitated chiefly by calcium and magnesium ions and

other ions such as aluminum, iron, manganese, strontium, zinc and by hydrogen ions. Since calcium and magnesium are present in significant amount in natural water, so hard ness is defined as a characteristic of water that represents the total concentration of just the calcium and magnesium ions expressed as mg l^{-1} calcium carbonate. Ethylenediami ne tetra acetic acid and di sodium salts, EDTA, form a chelated soluble complex when added to a solution of certain metal cations. If a small amount of a dye such as Eriochrom blackT is added to complex all the Mg and Ca ions, the solution will t urn from wi ne red to blue. This is the end point of titration. When EDTA is added to water containing both calcium and magnesium it combines first with the calcium. Calcium can be determined directly, using EDTA, when the PH is made sufficiently high, at pH of 12-13 that the magnesium is largely precipitated as the hydroxide and an indicator is used that combines with calcium only, Ethylenediaminetetraacetic acid and its sodium salts form a chelated soluble complex when added to a solution of certain metal cations. If a small amount of a dye such as Eriochrome Black T or Calmagite is added to an aqueous solution containing calcium and magnesium ions at a pH of 10.0±0.1, the solution becomes wine red. If EDTA is added as a titrant, the calcium and magnesium will be complexed, and when all of the magnesium and calcium has been complexed the solution turns from wine red to blue, marking the end point of the titration. Magnesium ion must be present to yield a satisfactory end point. To insure this, a small amount of complexometrically neutral magnesium salt of EDTA is added to the buffer; this automatically introduces sufficient magnesium and obviates the need for a blank correction. The sharpness of the end point increases with increasing pH. However, the pH cannot be increased indefinitely because of the danger of precipitating calcium carbonate, $CaCO_3$, or magnesium hydroxide, $Mg(OH)_2$, and because the dye changes color at high pH

values. The specified pH of 10.0±0.1 is a satisfactory compromise. A limit of 5 min is set for the duration of the titration to minimize the tendency toward $CaCO_3$ precipitation.

5-5- Apparatus and Reagent

a. Buffer solution: Dissolve 16.9 g ammonium chloride (NH_4Cl) in 143 mL conc ammonium hydroxide (NH_4OH). Add 1.25 g magnesium salt of EDTA (available commercially) and dilute to 250 mL with distilled water.

If the magnesium salt of EDTA is unavailable, dissolve 1.179 g disodium salt of ethylenediaminetetraacetic acid dihydrate (analytical reagent grade) and 780 mg magnesium sulfate ($MgSO_4 \cdot 7H2O$) or 644 mg magnesium chloride ($MgCl_2 \cdot 6H_2O$) in 50 mL distilled water. Add this solution to 16.9 g NH_4Cl and 143 mL conc NH_4OH with mixing and dilute to 250 mL with distilled water. To attain the highest accuracy, adjust to exact equivalence through appropriate addition of a small amount of EDTA or MgSO4 or $MgCl_2$. Store Solution or in a plastic or borosilicate glass container for no longer than 1 month. Stopper tightly to prevent loss of ammonia (NH_3) or pickup of carbon dioxide (CO_2). Dispense buffer solution by means of a bulb-operated pipet. Discard buffer when 1 or 2 mL added to the sample fails to produce a pH of 10.0 ± 0.1 at the titration end point. Satisfactory alternate ‘‘odorless buffers’’ also are available commercially. They contain the magnesium salt of EDTA and have the advantage of being relatively odorless and more stable than the NH_4Cl-NH_4OH buffer. They usually do not provide as good an end point as NH_4Cl-NH_4OH because of slower reactions and they may be unsuitable when this method is automated. Prepare one of these buffers by mixing 55 mL conc HCl with 400 mL distilled water and

then, slowly and with stirring, adding 300 mL 2-aminoethanol (free of aluminum and heavier metals). Add 5.0 g magnesium salt of EDTA and dilute to 1 L with distilled water.

b. Indicators: Many types of indicator solutions have been advocated and may be used if the analyst demonstrates that they yield accurate values. The prime difficulty with indicator solutions is deterioration with aging, giving indistinct end points. For example, alkaline solutions of Eriochrome Black T are sensitive to oxidants and aqueous or alcoholic solutions are unstable. In general, use the least amount of indicator providing a sharp end point. It is the analyst's responsibility to determine individually the optimal indicator concentration.
c. Eriochrome Black T: Sodium salt of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid; No. 203 in the Color Index. Dissolve 0.5 g dye in 100 g 2,2′,2″-nitrilotriethanol (also called triethanolamine) or 2-methoxymethanol (also called ethylene glycol monomethyl ether). Add 2 dr.
d. Standard EDTA titrant, 0.01M: Weigh 3.723 g analytical reagent-grade disodium ethylenediamine tetra acetatedihydrate, also called (ethylenedinitrilo)tetra acetic acid disodium salt (EDTA), dissolve in distilled water, and dilute to 1000 mL.ops per 50 mL solution to be titrated. Adjust volume if necessary.
e. Standard calcium solution: Weigh 1.000 g anhydrous CaCO3 powder (primary standard or special reagent low in heavy metals, alkalis, and magnesium) into a 500-mL erlenmeyer flask. Place a funnel in the flask neck and add, a little at a time, 1 + 1 HCl until all CaCO3 has dissolved. Add 200 mL distilled water and boil for a few minutes to expel C_{O2}. Cool, add a few drops of methyl red indicator, and adjust to the intermediate orange color by adding 3N NH4OH or 1

+ 1 HCl, as required. Transfer quantitatively and dilute to 1000 mL with distilled water; 1 mL = 1.00 mg $CaCO_3$.

Murexide (ammonium purpurate) indicator: This indicator changes from pink to purple at the end point. Prepare by dissolving 150 mg dye in 100 g absolute ethylene glycol. Water solutions of the dye are not stable for longer than 1 d. A ground mixture of dye powder and sodium chloride (NaCl) provides a stable form of the indicator. Prepare by mixing 200 mg murexide with 100 g solid NaCl and grinding the mixture to 40 to 50 mesh. Titrate immediately after adding indicator because it is unstable under alkaline conditions. Facilitate end-point recognition by preparing a color comparison blank containing 2.0 mL NaOH solution, 0.2 g solid indicator mixture (or 1 to 2 drops if a solution is used), and sufficient standard EDTA titrant (0.05 to 0.10 mL) to produce an unchanging color.

f. Sodium hydroxide, NaOH, 0.1N.
g. Magnetic Stirrer
h. Balance

Figure1.pH meter

Figure2. Magnetic stirrer

Figure3. Balance

5-6- Procedure

5-6-1- Total Hardness

Titration of sample: Select a sample volume that requires less than 15 mL EDTA titrant and complete titration within 5 min, measured from time of buffer addition. Dilute 25.0 mL sample to about 50 mL with distilled water in a porcelain casserole or other suitable vessel. Add 1 to 2 mL buffer solution. Usually 1 mL will be sufficient to give a pH of 10.0 to 10.1.

Add 1 to 2 drops indicator solution or an appropriate amount of dry-powder indicator formulation. Add standard EDTA titrant slowly, with continuous stirring, until the last reddish tinge disappears. Add the last few drops at 3- to 5-s intervals. At the end point the solution normally is blue.

5-6-2- Calcium

Add 2.0 mL NaOH solution or a volume sufficient to produce a pH of 12 to 13. Stir. Add 0.1 to 0.2 g indicator mixture selected (or 1 to 2 drops if a solution is used). Add EDTA titrant slowly, with continuous stirring to the proper end point. When using murexide, check end point by adding 1 to 2 drops of titrant in excess to make certain that no further color change occurs. color changes from pink to purple at the end point.

5-7 Calculation:

$$\text{Hardness (EDTA) as mg } CaCO_3/L = \frac{A \times B \times 1000}{\text{mL sample}}$$

where:

A = ml titration for sample and

B = mg CaCO3 equivalent to 1.00 mL EDTA titrant.

$$mg\ Ca/L = \frac{A \times B \times 400.8}{mL\ sample}$$

$$\text{Calcium hardness as mg } CaCO_3/L = \frac{A \times B \times 1000}{mL\ sample}$$

where:

A = ml titrant for sample and

B = mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant at the calcium indicator end

5-8- Question

I) Calculate the amount of Mg present in water sample.

II) What are the methods of permanent hardness reduction?

Sulfate ($SO4^{2-}$)

6-1- Purpose of the experiment:

In this experiment sulfate is determined in concentration range ofl 1-60 mg lit^{-1} colorimetric method.

6-2- Outcome of experiment

During this experiment, students learn that the sulfate ion of water is measured in the water laboratory using the turbidimeter method and the spectrophotometer. Sulfate is scattered in nature and its concentration in water ranges from small amounts to several hundred milligrams per liter. Calcium and magnesium sulfates in water increase hardness in water. From sewage drainage, a significant amount of sulfate enters the water through oxidation, which is not suitable for the environment and must be removed or reduced.

6-3- Application of concepts in industry

Ammonium sulfate, which is also called sodium salt of sulfuric acid, is a chemical substance that used in agricultural fertilizer, food, textile, water treatment and medical field. It can be extracted from natural salts or lakes with high salinity content. It is used in water purification, petrochemical industry, pharmaceutical industry, paper production, production of detergents and cleaners, textile and dyeing industry, and mines. In addition to the above, aluminum sulfate is used in wastewater treatment and in the production of cosmetics. Sulfate ion stabilizes the water-in-oil emulsion that is used in oil tanks.

6-4- Theory of Experiment

Sulfate is widely distributed in nature and may be present in natural waters in concentrations ranging from a few to several thousand milligrams per liter. Mine drainage wastes may contribute large amounts of $SO4^{2-}$ through pyrite oxidation. Sodium and magnesium sulfate exert a cathartic action. Sulfate ion, SO_4^{2-}, is precipitated in an acetic acid medium with barium chloride, $BaCl_2$, so as to form barium sulfate, $BaSO_4$, crystals of uniform size. Light absorbance of the $BaSO_4$ suspension is measured by a photometer and the $SO4^{2-}$ concentration is determined by comparison of the reading with a standard curve.

6-5- Apparatus and Reagent

a. Magnetic stirrer.
b. Photometer: One of the following is required, with preference in the order given:
   Nephelometer.
   Spectrophotometer, for use at 420 nm, providing a light path of 2.5 to 10 cm.
c. Filter photometer, equipped with a violet filter having maximum transmittance near 420 nm and providing a light path of 2.5 to 10 cm.
d. Stopwatch or electric timer.
e. Measuring spoon, capacity 0.2 to 0.3 mL
f. Barium chloride, $BaCl_2$, crystals, 20 to 30 mesh. In standardization, uniform turbidity is produced with this mesh range and the appropriate buffer.
g. Standard sulfate solution: Prepare a standard sulfate solution as described, 1.00 mL = 100 µg $SO4^{2-}$. Dilute 10.4 mL standard 0.0200N H_2SO_4 titrant specified in Alkalinity.

h. Conditioning reagent: Distilled water 300 ml, Hydrochloric Acid 60 ml, Glycerol 30 ml, Ethanol or Iso propil Alcohol 50 ml, Sodium Chloride 75 g.

Figure1. spectrophotometer

Figure2. Magnetic stirrer

Figure3. Balance

6-6- Procedure

Measure 100 ml sample or a suitable portion made up to I 00 ml, into a 250 ml Erlenmeyer flask. Add exactly 5 ml conditioning reagent and mix by the magnetic stirrer. While the solution is being stirred add a spoonful of $BaCl_2$ crystals and begin timing immediately. Stir for exactly 1 min at a constant speed.

After the stirring period had ended, pour some of the solution into the absorption cell of the photometer and measure the turbidity at 30 sec intervals for 4 minute. Record the maximum turbidity reading in the 4 minute intervals.

Estimate the sulfate concentration in the sample by comparing the turbidity reading with a calibration curve prepared by carrying sulfate standards. Through the entire procedure prepare standard sulfate solution in concentration range from 0 to 40 mg/lit sulfate.

6-7- Calculation

Calculate the sulfate concentration by:

$$\text{mg } SO_4^{2-}/\text{L} = \frac{\text{mg } SO_4^{2-} \times 1000}{\text{mL sample}}$$

6-8- Question

I) Is there other method for determination of Sulfate? Explain

II) What are the Interferances in this method?

Jar Test

7-1- Purpose of Experiment

Suspended matter in raw water is removed by clarification processes in three steps including Coagulation, Flocculation and Sedimentation. In this experiment the clarification process is to be considered and the effect of different coagulant with different concentration is to be studied.

7-2- Outcome of Experiment

During this experiment, students learn that One of the wastewater treatment methods is the use of coagulants in the jar test to remove colloidal substances. In this case, the importance of doing the tartest test becomes clear. To use the coagulation and flocculation method, it is necessary to perform the jartest. Jartest is a simple, low-cost and fast method for simulating the performance of coagulants in the water and wastewater treatment process. This system is efficient for calculating the relative approval of different coagulants, optimal time and interference, and different speeds. Also other influencing factors such as pH, temperature, stirring speed, type and how to add chemicals based on water analysis.

7-3- Application of concepts in industry

Jartest is used to show the effect of chemicals in treatment plants, which according to this test is a general method for evaluating coagulation, flocculation and sedimentation in treatment plants. The jar test is actually a small model of rapid mixing, coagulation and sedimentation units in the treatment plant, in which the

addition of chemicals, especially materials such as alum, chlorferric, and polymers, which are used to reduce water turbidity, can be evaluated on a laboratory scale. By using the jar test, the operator can roughly determine the amount of injection of complexing materials when the values of turbidity, color and other indicators of raw water quality change, and according to the results obtained from this test, the amount of injection of chemicals in the treatment Adjust the house. Of course, usually based on experience, managers may add a few percentages to the amount obtained in theory.

7-4- Theory of Experiment

Finely divided particles suspended in surface water repel each other because their surfaces are predominantly negatively charged. Clarification involves the following steps to ellow the particles to agglomerate:

Use of inorganic salts of aluminum or iron which neutralize the charge on the turbidity particles. These also hydrolyze to form insoluble precipitates which entrap additional particles.

Use of water–soluble organic polymers with numerous ionized sites for coagulation and flocculation.

Coagulation, therefore, involves neutralizing charges destabilize suspended solids. Flocculation starts when neutralize entrapped particles begin colliding and growing in size and form a large agglomeration or floc. Sedimentation occurs once the particles have been coagulated and flocculated

7-5- Apparatus and Reagent

a. Jartesting apparatus
b. Turbidimeter apparatus
c. Aluminum sulfate
d. Pac
e. Iron(ii) sulfate
f. Iron (iii) Chloride

Figures 1, turbidimeter apparatus

Figures 2, Jartesting apparatus

7-6- Procedure

Fill the Jartesting apparatus container with sample water. Add different coagulant dosage to each container to determine optimum operating conditions. Stir at approximately 100 rpm for one minute. The rapid mix stage helps to disperse the coagulant throughout each container. Reduce the stirring speed to 25 t0 35 rpm and

continue mixing for 15 to 20 minutes. The slow mixing helps to promote floc formation by enhancing particle collisions which leads to larger flocs. Turn off the mixer and ellow the containers to settle for 30 to 45 minutes. Then measure the final turbidity.

7-7- Calculation

This experiment doesn't have any formula. Just needs to compare the datas.

7- 8- Question

I) Compare the datas and determine that witch coagulant is better for the reduce of turbidity.

II) Plot the turbidity versus coagulant dose.

III) Ditermine the optimum dose and discuss about them.

Chemical Oxygen Demand (COD)

8-1- Purpose of Experiment

Perpose of COD Experiment is The value of COD equivalent to the amount of oxygen that is required for the oxidation of organic substances using potassium dichromate in the presence of silver sulfate salts as a reaction catalyst in an acidic environment.

8-2- Outcome of Experiment

During this experiment, students learn that the amount of pollution in water and wastewater is measured by the COD test, in other words chemical oxygen demand, which is one of the fastest methods in the study and control of wastewater treatment systems and can lead us to get results and also, the student gets acquainted with various chemical equipments and reagents to do this work.

8-3- Application of concepts in industry

One of the most important steps in designing a sanitary and industrial wastewater treatment package is to identify the type of wastewater and the pollutants in it. Finally, based on this, effective methods to reduce pollution are identified and implemented with the required equipment in the package. By measuring the COD of wastewater, one can actually find out several important issues, the most important of which are:

Determining the required methods for wastewater treatment and reducing the amount of chemicals in them.

Checking the efficiency of equipment and purification processes used in the package or purification ponds.

This method is the best option for factory effluent monitoring, effluent treatment (input and output water), detection of organic leakage and product loss (for example in chemical, petrochemical and food plants).

8-4- Theory of Experiment

Chemical oxygen demand is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. Because of its unique chemical properties, the dichromate ion, $Cr_2O_7^{2-}$, is the specified oxidant in Methods. it is reduced to the chromic ion, Cr^{3+}, in these tests. Both organic and inorganic components of a sample are subject to oxidation, but in most cases the organic component predominates and is of the greater interest. If it is desired to measure either organic or inorganic COD alone, additional steps not described here must be taken to distinguish one from the other.COD is a defined test; the extent of sample oxidation can be affected by digestion time, reagent strength, and sample COD concentration. COD often is used as a measurement of pollutants in wastewater and natural waters. Other related analytical values are biochemical oxygen demand[1], total organic carbon[2], and total oxygen demand[3]. In many cases it is possible to correlate two or more of these values for a given sample. BOD is a measure of oxygen consumed by microorganisms under specific conditions; TOC is a measure

[1] BOD
[2] TOC
[3] TOD

of organic carbon in a sample; TOD is a measure of the amount of oxygen consumed by all elements in a sample when complete (total) oxidation is achieved.

COD is done in two ways that includes (COD Open Reflux) and (COD Closed Reflux)

8-5-1 Apparatus and Reagent (COD Open Reflux)

a. reflux apparatus, consisting of 500- or 250-mL erlenmeyer flasks with ground-glass 24/40 neck and 300-mm jacket Liebig, West, or equivalent condenser with 24/40 ground-glass joint, and a hot plate having sufficient power to produce at least 1.4 W/cm2 of heating surface, or equivalent.
b. Blender. (Magnetic stirrer)
c. Balance
d. Pipets, Class A and wide-bore.
e. Standard potassium dichromate solution, 0.04167M: Dissolve 12.259 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, in distilled water and dilute to 1000 mL. This reagent undergoes a six-electron reduction reaction; the equivalent concentration is 6 × 0.04167M or 0.2500N.
f. Sulfuric acid reagent: Add Ag_2SO_4, reagent or technical grade, crystals or powder, to conc H2SO4 at the rate of 5.5 g Ag_2SO_4 /kg H_2SO_4. Let stand 1 to 2 d to dissolve. Mix.
g. Ferroin indicator solution: Dissolve 1.485 g 1,10-phenanthroline monohydrate and 695 mg $FeSO_4 \cdot 7H_2O$ in distilled water and dilute to 100 mL. This indicator solution may be purchased already prepared.

h. Standard ferrous ammonium sulfate (FAS) titrant, approximately 0.25M: Dissolve 98

i. $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 mL conc H_2SO_4, cool, and dilute to 1000 mL. Standardize this solution daily against standard $K_2Cr_2O_7$ solution as follows:

Dilute 25.00 mL standard $K_2Cr_2O_7$ to about 100 mL. Add 30 mL conc H_2SO_4 and cool. Titrate with FAS titrant using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.

$$\text{Molarity of FAS solution} = \frac{\text{Volume 0.04167M } K_2Cr_2O_7 \text{ solution titreated, ml}}{\text{Voiume FAS used in titration, mL}} \times 0.2500$$

j. Mercuric sulfate, $HgSO_4$, crystals or powder.

k. Sulfamic acid: Required only if the interference of nitrites is to be eliminated.

l. Potassium hydrogen phthalate (KHP) standard, $HOOCC_6H_4COOK$: Lightly crush and then dry KHP to constant weight at 110°C. Dissolve 425 mg in distilled water and dilute to 1000 mL. KHP has a theoretical COD1of 1.176 mg O_2 /mg and this solution has a theoretical COD of 500 µg O_2 / mL. This solution is stable when refrigerated, but not indefinitely. Be alert to development of visible biological growth. If practical, prepare and transfer solution under sterile conditions. Weekly preparation usually is satisfactory.

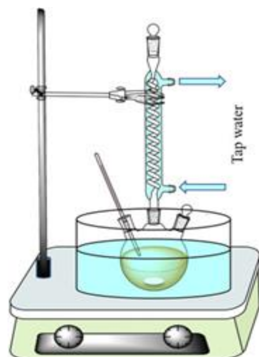

Figure1.schematic of Reflax system

Figure2. Magnetic stirrer

Figure3. Balance

8-5-2-Apparatus and Reagent (COD Closed Reflux)

a. Magnetic stirrer

b. Balance

c. COD reactor. Ensure that reaction vessels are of optical quality. Other types of absorption cells with varying path lengths may be used. Use the extinction coefficients of the ions of interest for this approach.

d. Spectrophotometer, for use at 600 nm and/or 420 nm with access opening adapter for ampule or 16-, 20-, or 25-mm tubes. Verify that the instrument operates in the region of 420 nm and 600 nm. Values slightly different from these may be found, depending on the spectral bandpass of the instrument.

e. Digestion solution, high range: Add to about 500 mL distilled water 10.216 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, 167 mL conc H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 mL.

f. Digestion solution, low range: Prepare as in 3a, but use only 1.022 g potassium dichromate.

g. Sulfuric acid reagent: Sulfuric acid reagent: Add Ag_2SO_4, reagent or technical grade, crystals or powder, to conc H2SO4 at the rate of 5.5 g Ag_2SO_4 /kg H_2SO_4. Let stand 1 to 2 d to dissolve. Mix

h. Sulfamic acid: Required only if the interference of nitrites is to be eliminated.

i. Potassium hydrogen phthalate standard: Lightly crush and then dry KHP to constant weight at 110°C. Dissolve 425 mg in distilled water and dilute to 1000 mL. KHP has a theoretical COD1 of 1.176 mg O_2 /mg and this solution has a theoretical COD of 500 µg O_2 / mL. This solution is stable when refrigerated, but not indefinitely. Be alert to development of visible biological growth. If practical, prepare and transfer solution under sterile conditions. Weekly preparation usually is satisfactory.

Figure 1. COD reactor Figure 2. Spectrophotometer

8-6-1 Procedure (COD Open Reflux)

Treatment of samples with COD of >50 mg O2 /L: Blend sample if necessary and pipet 50.00 mL into a 500-mL refluxing flask. For samples with a COD of >900 mg O2 /L, use a smaller portion diluted to 50.00 mL. Add 1 g $HgSO_4$, several glass beads, and very slowly add 5.0 mL sulfuric acid reagent, with mixing to dissolve $HgSO_4$. Cool while mixing to avoid possible loss of volatile materials. Add 25.00 mL 0.04167M $K_2Cr_2O_7$ solution and mix. Attach flask to condenser and turn on cooling water. Add remaining sulfuric acid reagent (70 mL) through open end of condenser. Continue swirling and mixing while adding sulfuric acid reagent. CAUTION: Mix reflux mixture thoroughly before applying heat to prevent local heating of flask bottom and a possible blowout of flask contents. Cover open end of condenser with a small beaker to prevent foreign material from entering refluxing mixture and reflux for 2 h. Cool and wash down condenser with distilled water. Disconnect reflux condenser and dilute mixture to about twice its volume with distilled water. Cool to room temperature and titrate excess $K_2Cr_2O_7$ with FAS, using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator. Although the quantity of ferroin indicator is not critical, use the same volume for all titrations. Take as the end point of the titration the first sharp color change from blue-green to reddish brown that persists for 1 min or longer. Duplicate determinations should agree within 5% of their average. Samples with suspended solids or components that are slow to oxidize may require additional determinations. The blue-green may reappear. In the same manner, reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of sample.

8-6-2- Procedure (COD Closed Reflux)

Treatment of samples: Measure suitable volume of sample and reagents into tube or ampule. Prepare, digest, and cool samples, blank. Note the safety precautions. It is critical

that the volume of each component be known and that the total volume be the same for each reaction vessel. If volumetric control is difficult, transfer digested sample, dilute to a known volume, and read. Premixed reagents in digestion tubes are available commercially.

Measurement of dichromate reduction: Cool sample to room temperature slowly to avoid precipitate formation. Once samples are cooled, vent, if necessary, to relieve any pressure generated during digestion. Mix contents of reaction vessels to combine condensed water and dislodge insoluble matter. Let suspended matter settle and ensure that optical path is clear. Measure absorption of each sample blank and standard at selected wavelength (420 nm or 600 nm). At 600 nm, use an undigested blank as reference solution. Analyze a digested blank to confirm good analytical reagents and to determine the blank COD; subtract blank COD from sample COD. Alternately, use digested blank as the reference solution once it is established that the blank has a low COD. At 420 nm, use reagent water as a reference solution. Measure all samples, blanks, and standards against this solution. The absorption measurement of an undigested blank containing dichromate, with reagent water replacing sample, will give initial dichromate absorption. Any digested sample, blank, or standard that has a COD value will give lower absorbance because of the

decrease in dichromate ion. Analyze a digested blank with reagent water replacing sample to ensure reagent quality and to determine the reagents' contribution to the decrease in absorbance during a given digestion. The difference between absorbances of a given digested sample and the digested blank is a measure of the sample COD. When standards are run, plot differences of digested blank absorbance and digested standard absorbance versus COD values for each standard.

Preparation of calibration curve: Prepare at least five standards from potassium hydrogen phthalate solution with COD equivalents to cover each concentration range. Make up to volume with reagent water; use same reagent volumes, tube, or ampule size, and digestion procedure as for samples. Prepare calibration curve for each new lot of tubes or ampules or when standards prepared in differ by ≥5% from calibration curve. Curves should be linear. However, some nonlinearity may occur, depending on instrument used and overall accuracy needed.

8-7-1 Calculation (COD Open Reflux)

$$\text{COD as mg } O_2/L \; \frac{(A-B)\times M\times 8000}{\text{mL sample}}$$

where:

A = mL FAS used for blank,

B = mL FAS used for sample,

M = molarity of FAS,

and 8000 = milliequivalent weight of oxygen × 1000 mL/L.

8-7- 2- Calculation (COD Closed Reflux)

The results of COD are read by using a spectrophotometer with a standard graph drawn.

8- 8- Question

I) Is there a difference between COD and BOD tests?

II) What is the COD closed reflux test vial and what is its use?

Water Microbiology

9-1- Purpose of Experiment

Observe the colony of bacteria in water and wastewater by different solid culture.

9-2- Outcome of Experiment

In this experiment, the student learns how to make a bacterial culture medium and the methods of bacterial culture in a solid medium, and also learns about water pollution with the growth of water bacteria and the formation of its colony.

9-3- Application of concepts in industry

In fact, microbiology was created with the aim of identifying microscopic organisms and various issues related to their life, and it is the basis of many sciences, including biotechnology, biochemistry, genetics, and medicine. Regarding the application of microbiology in the oil industry, two aspects can be considered as quantitative improvement and qualitative improvement. Biocide or antibacterial drilling mud, anti-algae, anti-bacterial are used to maximize production by inhibiting biofilm and corrosion caused by microbial load. Biocide is used in small amounts to control the growth of bacteria and other harmful organisms in oil wells. And most importantly, it plays a fundamental role in the water and wastewater treatment industry, especially drinking water.

9-4- Theory of Experiment

Bacteria are common single-celled organisms and are a natural component of lakes, rivers, and streams. Most of these bacteria are harmless to humans; however, certain bacteria, some of which normally inhabit the intestinal tract of warm-blooded animals, have the potential to cause sickness and disease in humans. High numbers of these harmless bacteria often indicate high numbers of harmful bacteria as well as other disease-causing organisms such as viruses and protozoans.

One method of determining bacteria counts is to count the number of bacteria colonies that grow on a prepared medium. Escherichia coli (abbreviated as E. coli) are bacteria found in the environment, foods, and intestines of people and animals. E. coli are a large and diverse group of bacteria. Although most strains of E. coli are harmless, others can make you sick. Some kinds of E. coli can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses.

The most important bacteria in water are total coliform, facal coliformand E. coli

9-4-1- Total coliform

Total coliforms are gram-negative, aerobic or faculative anaerobic, nonspore forming rods. These bacteria were originally believed to indicate the presence of fecal contamination, however total coliforms have been found to be widely distributed in nature and not always associated with the gastrointestinal tract of warm blooded animals. The number of total coliform bacteria in the environment is still widely used as an indicator for potable water in the U.S.

9-4-2- Fecal coliform

Fecal coliform bacteria are a subgroup of coliform bacteria that were used to establish the first microbial water quality criteria. The ability to grow at an elevated temperature (44.5 degrees Celsius) separate these bacteria from the total coliforms and make it a more accurate indicator of fecal contamination by warm-blooded animals. Fecal- coliform bacteria are detected by counting the dark-blue to blue-grey colonies that grow on a 0.65 micron filters placed on MFC agar incubated in a 44.5º C oven for 22-24 hours. The presence of fecal coliforms in water indicates that fecal contamination of the water by a warm-blooded animal has occurred, however, recent studies have found no statistical relationship between fecal coliform concentrations and swimmer-associated sickness.

9-4-3- E. coli

Escherichia coli (E. coli) is a rod-shaped bacterium commonly found in the gastrointestinal tract and feces of warm-blooded animals. It is a member of the fecal coliform group of bacteria and is distinguished by its inability to break down urease. E. coli numbers in freshwater are determined by counting the number of yellow and yellow brown colonies growing on a 0.45-micron filter placed on m-TEC media and incubated at 35.0º C for 22-24 hours. The addition of urea substrate confirms that colonies are E. coli. This bacterium is a preferred indicator for freshwater recreation and its presence provides direct evidence of fecal contamination from warm-blooded animals. Although usually harmless, E. coli can cause illnesses such as meningitis, septicemia, urinary tract, and intestinal infections. A recently discovered strain of E. coli (E. coli 0157:H7) can cause severe disease and may be fatal in small children and the elderly. The relation between bacteria counts and sickness Consumption of

or contact with water contaminated with feces of warm-blooded animals can cause a variety of illnesses. Minor gastrointestinal discomfort is probably the most common symptom; however, pathogens that may cause only minor sickness in some people may cause serious conditions or death in others, especially in the very young, old, or those with weakened immunological systems.

9-4-4- Classification of alive beings

Monera (bacteria&cyanobacter), Protista (unicellular), Fungi, Planta, Animalia, Organisms have other divisions that include eukaryotes and prokaryotes, that all of are eukaryotes. except Monera.

Bacteria are a group of microscopic unicellular organisms that have a simple structure and belong to the prokaryotes group. They are surrounded by a relatively thick outer covering. Bacteria are the most diverse and important microorganisms. Few of them are pathogenic in humans, animals and plants. In general, without their activity, life on the planet will be disrupted. Certainly, eukaryotes originated from living organisms like bacteria, considering that bacteria have a simple structure and many of them can be easily grown and controlled in the laboratory.

9-4-5- the characteristics of bacteria

Based on morphology: ROD (rod) - Cocci (round) - Filametus (elongated spiral)

In terms of the need for oxygen consumption:

Obligate aerobe: they are 100% aerobic.

Obligate anaerobe: They are 100% anaerobic and do not need oxygen or air for their growth. And basically the air is poisonous for them.

Facultative anaerobes: able to grow both in aerobic and anaerobic conditions.

microaerophilic: they need 5% and 10% carbon dioxide and cannot tolerate much oxygen.

capnophilic: these need carbon dioxides in the culture medium.

Bacteria are divided into three categories according to their energy source.

Chemotrophic b: These Bacteria get the energy they need from chemical reactions. That is, the source of energy is non-light, the bacteria in this category are divided into two parts:

Chemorganotrophicb: these bacteria obtain their energy from the oxidation or fermentation of organic materials.

Chemo lithotrophic: They gain energy by oxidizing minerals (inorganic) and regenerating CO_2.

Phototrophic bacteria:

This group of bacteria obtain energy with the help of sunlight and chemical reactions and are divided into two categories:

photoorganotrophs and photolithotrophs.

Paratrophic bacteria:

this category takes energy from the host cell, such as rickettsiae, chlamydiae and viruses, that is, this category includes bacteria that are obligate parasites.

From the point of Nutrient Requierment, bacteria are divided into two categories:

Hetrotroph: This category needs one or more ready-made substances to meet nutritional needs, such as some vitamins or growth factors or some carbon sources.

Autotroph: This group of bacteria synthesizes all their nutritional needs from organic and inorganic materials.

Bacteria in terms of temperature requirements:

Sacrophilic bacteria that grow between 0°C and 20°C and their optimal temperature is 10°C.

Mesophilic bacteria that grow in a temperature range between 10°C and 52°C, and the optimum temperature is 37°C.

Thermophilic or thermophilic bacteria that grow at a temperature between 45°C and 80°C, but the best growth is at a temperature of 55°C.

Bacteria in terms of pH tolerance

Acidophiles that tend to pH below 7 and grow more at this pH. The optimal pH for this category of bacteria is 6 – 6.5.

Alkalinophiles grow better in the pH range between 8.5 and 9.

Neutrophils need a neutral pH, that is, 7 to 7.2 for growth

bacteria in term of Gram: G^+ and G^-

B G^+: If the bacteria on the slide is stained with an alkaline dye such as crystal violet and iodine solution, and after washing with alcohol or acetone (stain), it takes on a blue color, the reason is the peptidoglycan layer in the cell wall that is thicker.

B G^-: If the bacterra is stained with safranin or Fushin Fenike. the bacteria takes on a red color, the cause is another layer called the outer membrane, which is a phospholipid membrane.

9-4-6- Bacteria Structure

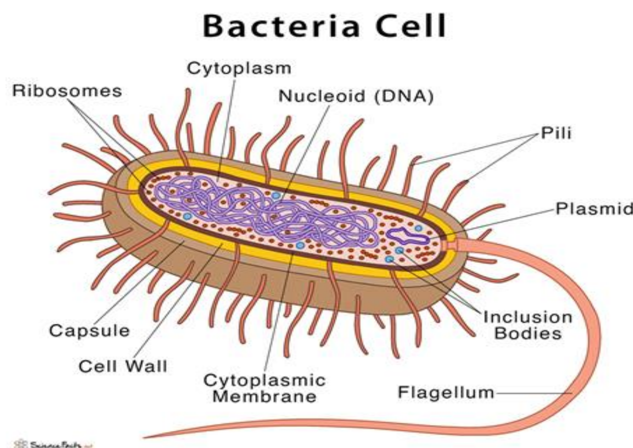

Figure1. schematic of Bacteria Structure

Flagellum: It is made up of protein units called flagellin and can be repaired and is the means of bacterial movement. Its length is usually several times the length of the bacteria. which can be seen as single flagella, two flagella and surface multi-flagella.

The cell wall: the wall is outside the plasma membrane and causes the strength of the bacteria and it gives shape to bacteria

Cytoplasmic membrane: It is a thin membrane inside the bacterial wall and consists of fat and protein molecules.

Ribosome: They are part of the cytoplasm, the storage material of the chromatomere, the genetic material, whose DNA is often folded and compacted in the form of a chromosome.

Pili: An appendage that is seen on the surface of bacteria, is short and has a cilia-like structure. Some of these excesses are involved in the attachment of bacteria to different surfaces, as well as some in conjugation and DNA transfer from one bacterium to another.

Mesosome: It is obtained from the indentation of the cytoplasmic membrane into the cytoplasm and is often present at the dividing wall. And they are involved in the process of DNA division, protozoan division and the transformation of bacteria into spores.

9-4-7- Bacteria growth

Bacteria do not have the same speed in all stages of growth. While the bacteria is transferred from one environment to another and the growth conditions and requirements are met, the growth and multiplication of bacteria takes place during the following stages:

First stage: Adaptation in this stage, the number of bacteria does not change with the passage of time and the bacteria gets to know the new environment and fulfills its basic needs.

The second stage: the beginning of multiplication and growth, the number of bacteria increases over time, but the rate of increase is not high.

The third stage: It is the exponential growth stage in which the bacteria rapidly increase in number over time

Fourth stage: In this stage, the number of cells remains constant over time.

The fifth stage: which is the last stage, with the passage of time, the number of bacteria decreases, which is common to the death phase.

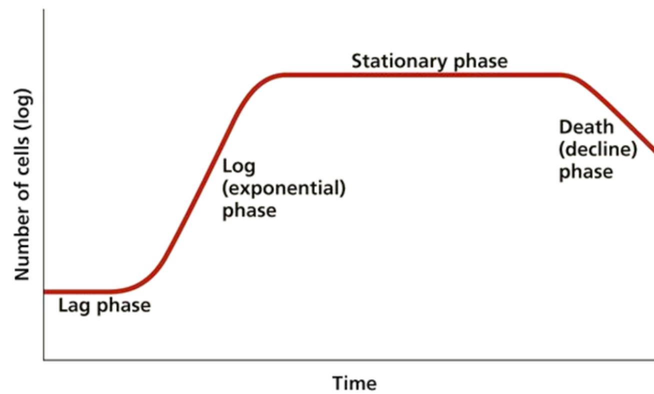

Figure 2, the growth steps of bacteria

9-5- Apparatus and Reagent

a. Outoclave

b. Incubator

c. Gas burner

d. Microbial Hood

e. Plate or petry dish

f. Metal loop

g. Culture medium Like (Nutrient agar and EMB)

Figure3. Outoclave Figure4. Incubator Figure5. Gas burner

Figure6: Microbial Hood Figure7: Balance Figure8:Manetic stirrer

9-6- Procedure

make the culture medium according to the instructions that written on the box. Pour the prepared medium in the plate and cool it. then open the plate beside the flame, because the medium shouldn't be polluted. Take the loop on the flame until become. Then cool the loop beside the flame. Put the loop in the sample (water) and make

transfer to the culture medium. Do streak culture and close the plate. Keep in incubator for 48 hours. After that time, you can observe the colony of bacteria.

9-7- Calculation

This experiment doesn't have any formulas for calculate.

9-8- Question

I. What are photosynthetic bacteria that may grow in sewage stabilization ponds? Describe their function.
II. The presence of coliform organisms in water indicates what issue in drinking water

Refrances:

- Standard Method for the Examination of Water and Wastewater (20th Edition)
- The Booklet of Microbiology (Translate persian to English)