Military Institute of Science and Technology (MIST)
Department of Civil Engineering (CE)

Environmental Engineering Laboratory

Course No: CE 332
Course Title: Environmental Engineering Lab
Course Credit: 1.50

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Experiment No. 1
Determination of pH of Water

INTRODUCTION

pH is a measure of the acid or alkaline condition of water. It is a way of expressing the hydrogen ion concentration, or more preciously, the hydrogen ion activity. pH is defined as follows:

$$pH = -\log \{H^+\} \quad (1)$$

where, $\{H^+\}$ is the concentration (or activity) of hydrogen ion (or proton) in moles per liter (M).

Water dissociates to form hydrogen ion (H^+) and hydroxyl ion (OH^-) according to the following equation:

$$H_2O = H^+ + OH^- \quad (2)$$

At equilibrium, we can write,

$$= \{H^+\}\{OH^-\} / \{H_2O\} \quad (3)$$

But, since concentration of water is extremely large (approximately 55.5 mol/L) and is diminished very little by the slight degree of ionization, it may be considered as a constant and its activity is taken as 1.0. Thus, Eq. 3 may be written as:

$$K_w = \{H^+\}\{OH^-\} \quad (4)$$

where, K_w = Equilibrium Constant

For pure water at 25°C, $K_w = 10^{-7} \times 10^{-7} = 10^{-14}$. This is known as the ion product of water or ionization constant for water. In other words, water (deionized or distilled water) at 25 ^{0}C dissociates to yield 10^{-7} mol/L of hydrogen ion (H^+) and 10^{-7} mol/L of hydroxyl ion (OH^-). Hence, according to Eq. 1, pH of deionized water is equal to 7.0.

The pH is usually represented by a scale ranging from zero to 14 with 7 being neutral. Most natural waters are slightly alkaline due to the presence of bicarbonate and less often carbonate. Water with pH outside the desirable neutral range may exhibit sour tastes and accelerate the corrosion of metallic plumbing fittings and hot water services. Aeration removes carbon dioxide and hence causes a rise in pH value.

ENVIRONMENTAL SIGNIFICANCE

A controlled value of pH is desired in water supplies, sewage treatment and chemical process plants. In water supply pH is important for coagulation, disinfection, water softening and corrosion control. In biological treatment of waste pH is the most significant. Organisms involved in treatment plants are operative within certain pH range. According to Bangladesh Environment Preservation Act (1997), drinking water standard for pH is 6.5 - 8.5.

THEORY

Measurement of pH is carried out either colorimetrically or electrometrically. The electrometric method is more expensive but accurate whereas colorimetric method is cheap but serious errors may result from presence of color, turbidity, high saline content, colloidal matter, free chlorine and various oxidants and reductants. For colorimetric determination, a wide variety of indicators are used to determine pH. In colorimetric measurement standard color discs or standard color solutions may be used.

REAGENT: Standard pH solution for calibration of pH meter.

APPARATUS: pH meter

PROCEDURE:

1. Perform calibration of the pH meter using standard pH solutions. The calibration procedure should depend on the pH range of interest.

2. Take about 100 ml of the sample in a beaker. Make sure not to agitate the sample in order to avoid exchange of gases between the sample and the atmosphere.

3. Insert pH meter in the sample. Allow some time for attainment of equilibrium. Turn on the pH meter and take reading.

ASSIGNMENT QUESTIONS

1. Discuss the environmental significance of pH.

2. Define pH in terms of hydrogen-ion (H^+) concentration and hydroxyl-ion (OH^-) concentration. A decrease in pH of one unit represents how much increase in hydrogen-ion concentration?
3. At 25°C, hydroxyl ion concentration of a solution is $10^{-5.7}$. Determine the pH of the solution.

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Experiment No. 2
Determination of Color of Water

INTRODUCTION

Pure water should not possess any color. Most water available to us are colored to some extent due to the presence of various impurities (i.e., iron and manganese in association with organic matter from decaying vegetation). Impurities may be in the colloidal form in water or it may be in suspended state. Color caused by dissolved and colloidal form of impurities is called true color and that caused by suspended matter, in addition to dissolved and colloidal matters, is called apparent color. Ground water may show color due to the presence of iron compound. Color intensity generally increases with an increase in pH. For this reason recording pH along with color is advised.

ENVIRONMENTAL SIGNIFICANCE

Colored water is not always harmful to man, but in most cases it is. Even if the water is not harmful, it is not preferred by people for aesthetic reasons. Also, disinfection by chlorination of waters containing natural organics (which produces colour) results in the formation of chloroform, other trihalomethanes, and a range of other chlorinated organics, leading to problems, which is a major concern in water treatment. So it is important to limit the color of water for domestic supplies. According to Bangladesh Environment Preservation Act (1997), drinking water standard for color is 15 units.

THEORY

Color is usually expressed in platinum-cobalt units (Pt-Co Units) which is based on the intensity of color produced by a solution of Platinum and Cobalt salts that approximate the yellow-brown color of natural waters. For easier comparison color discs are prepared to represent various units of color. The sample is compared with the color discs and the color is determined.

APPARATUS: Spectrophotometer (HACH)

PROCEDURE

To be shown in class

ASSIGNMENT QUESTIONS

(1) Discuss the environmental significance of color.

(2) Discuss briefly the causes of color in water. What wavelength of light was used in the measurement of color in the laboratory?

(3) Write down the methods that are commonly used for removing color from water and wastewater.

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Experiment No. 3
Determination of Turbidity of Water

INTRODUCTION

The term "turbid" is applied to water containing suspended matter that interferes with passage of light through the water or in which visual depth is restricted. *Turbidity* may be caused by a wide variety of suspended substances of various sizes ranging in size from colloidal to coarse particles, depending on the degree of turbulence. In rivers under flood conditions, most of the turbidity will be due to relatively coarse particles. Whereas in lakes and other waters existing under relatively quiescent conditions, most of the turbidity will be due to colloidal and extremely fine particles.

ENVIRONMENTAL SIGNIFICANCE

Turbidity is important for water supply engineers as turbid water is not aesthetically acceptable to people. There is always a fear among the people that turbid water may cause diseases. For filtration, turbid water is not suitable as it causes quick clogging of filter bed that necessitates the use of pre-treatment plant. Turbidity is also an important parameter in disinfection process. Disinfection is usually accomplished by means of chlorine, ozone, or chlorine dioxide. To be effective, there must be contact between the agent and the organisms to be killed. However, in cases in which turbidity is caused by municipal wastewater solids, many of the pathogenic organisms may be encased in the particles and protected from the disinfectant. Hence USEPA has placed a maximum level of *0.5* to 1.0 units of turbidity, depending on the disinfection process used, as the maximum amount allowable in public water supplies.
According to Bangladesh Environment Preservation Act (1997), drinking water standard for Turbidity is 10 NTU (Nephelometric turbidity unit).

STANDARD UNIT OF TURBIDITY

Because of the wide variety of materials that cause turbidity in natural waters, it has been necessary to use an arbitrary standard. The original standard chosen was

1 mg SiO_2/L = 1 unit of turbidity

and the silica used had to meet certain specifications as to particle size.
Standard suspensions of pure silica are not now used for measuring turbidity. They were used originally to calibrate the Jackson candle turbidimeter, the former standard instrument for turbidity measurement [results expressed as JTU (Jackson Turbidity Unit)]. This was a rather crude instrument in which the turbidity of a suspension was measured by the depth of suspension through which the outline of a flame from a standard candle just disappeared. The Jackson candle turbidimeter was removed as a standard procedure from the 17th edition of "Standard Methods", as it has generally been replaced in practice by more reliable, sensitive,

and easier to use instrument that depend upon the principle of nethelometry. Also silica as a standard reference material has been replaced by standardized preparations of formazin polymer. The formazin suspensions were first calibrated against the Jackson candle turbidimeter, and thus there is some relationship between turbidity measurements by the Jackson Candle Turbidimeter and nephelometry.

However, the Jackson candle turbidimeter measures the interference of light passage in a straight line while nephelometry measures the scattering of light from particles. Because of this basic difference in the phenomenon measured, results from the two different procedures on different suspensions can vary widely. In order to avoid any confusion this may cause, turbidity measurements by the standard nephelometry procedure are now reported in Nephelometric Turbidity Units (NTU). In additions to formazin standards, commercially available standards such as styrene divinylbenzene beads are also available.

THEORY

The current standard method for measurement of turbidity depends upon instruments that employ the principles of nephelometry. In this instrument, a light source illuminates the sample and one or more photoelectric detectors are used with a readout device to indicate the intensity of scattered light at right angle to the path of the incident light. It is customary to use a particular formazin polymer suspension as a standard, or commercially available preparations may also be used. When using formazin standard, 40 NTU are about equivalent to 40 Jackson candle turbidity units (JTU). Turbidities as low as 0.02 NTU can be determined by this procedure provided that water with sufficiently low turbidity can be obtained for use in instrument calibration. Samples with turbidities greater than 40 NTU are diluted with turbidity-free water until values within the range of 30 to 40 NTU are obtained. The turbidity is then determined by multiplying the measured by the dilution factor.

APPARATUS: DR LANGE Turbidimeter

PROCEDURE

(1) Ensure that the Turbidimeter has been standardized recently.
(2) Fill the clean sample cell with the water sample and place it in the sample cell holder. Cover the sample cell with the light shield. Turn on the switch and read the turbidity directly from the monitor.

ASSIGNMENT QUESTIONS

(1) Discuss the environmental significance of turbidity.
(2) Why turbidity is important in "filtration" and "disinfection" processes?
(3) Write down the methods that are commonly used for removing turbidity from water.

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Experiment No. 4
DETERMINATION OF TOTAL SOLIDS, DISSOLVED SOLIDS AND SUSPENDED SOLIDS

INTRODUCTION

Total Solids (TS) refer to the matter that remains as residue upon evaporation and drying at 103 to 105°C. Total solids (TS) includes total suspended solids (TSS) - the portion of total solids retained by a filter, and total dissolved solids (TDS) - the portion that passes through the filter. Solids are incorporated in water from its source (e.g., minerals with which it has been in contact). In potable water, most of the solids remain in dissolved form and consists of inorganic salts, small amounts of organic matter, and dissolved gases. The total dissolved solids content of potable water usually ranges from 20 to 1000 mg/L. Groundwater usually has higher dissolved solids.

ENVIRONMENTAL SIGMFICANCE

To determine whether water is suitable for domestic purpose, it is required to know how much solid it contains. According to Bangladesh Environment Preservation Act (1997), potable water should not contain more than 1000 mg/l of total dissolved solids (TDS). Water with high dissolved solids generally is of inferior palatability and may induce unfavourable physiological reaction in the user. Water high in suspended solids may be aesthetically unsatisfactory for such purposes as bathing.

THEORY

Water has a boiling point of 100°C. It may rise by a few degrees due to the presence of impurities. When water with solids is kept at 103 to 105°C for 24 hours, the water gets evaporated and the solids either in dissolved state or in suspended state remain as residue.

APPARATTUS

1. Beaker (150 mL)	2pcs.
2. Measuring cylinder (100 mL)	1 pc.
3. Funnel	1 pc.
4. Dropper	lpc.
5. Filter paper	2 pcs.

PROCEDURE

Total Solids:

(1) Take a clear dry glass beaker (which was kept at 103°C in an oven for 1 hour) of 150 ml. capacity and put appropriate identification mark on it. Weigh the beaker and note the weight.

(2) Pour 100 ml. of the thoroughly mixed sample, measured by the measuring cylinder, in the beaker.

(3) Place the beaker in an oven maintained at 103 ^{0}C for 24 hours. After 24 hours, cool the beaker and weigh. Find out the weight of solids in the beaker by subtracting the weight of the clean beaker determined in step (1).

(4) Calculate total solids (TS) as follows:

Total solids, TS (mg/l) = mg of solids in the beaker x 1000 / (volume of sample)

Dissolved Solids:

(1) Same as above (step 1 of total solids).

(2) Take a 100 ml. of sample and filter it through a double layered filter paper and collect the filtrate in a beaker.

(3) Then repeat the same procedure as in steps (3) and (4) of the *total solids* determination and determine the dissolved solids content as follows:

Total Dissolved Solids, TDS (mg/l) = mg of solids in the beaker x 1000 / (volume of sample)

Suspended Solids:

Total Suspended Solids, TSS (mg/l) = TS (mg/L) - TDS (mg/L)

ASSIGNMENT QUESTIONS

1. Discuss environmental significance of solid content in water.
2. How dissolved solids concentration can be estimated from measurement of specific conductance?
3. Explain why groundwater typically contains high total solids compared to surface water. Why is the situation reverse for suspended solids?

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Experiment No. 5
Determination of Carbon-dioxide of Water

INTRODUCTION

The total carbon dioxide in water is the sum of the free carbon dioxide and the carbon dioxide existing in the form of bicarbonate and carbonate ions. The presence of free carbon dioxide in water is caused by the decay of organic matter, dissolution of carbon dioxide from under ground sources, and the dissolution from the atmosphere. Since the carbon dioxide content of the normal atmosphere is quite low (less than 0.04%), this is not a source of appreciable carbon dioxide except where flue gases containing a high percentage of carbon dioxide are brought in relatively close contact with the water. Surface waters usually contain less than 10 ppm (mg/L) of free CO_2, while some ground water are found to contain free carbon dioxide exceeding 50 ppm (mg/L).

ENVIRONMENTAL SIGNIFICANCE

Corrosion is the principal difficulty caused by carbon dioxide. This gas on solution in water producing carbonic acid (Eq. 1) resulting in lowering of pH value and thus produces corrosive characteristic in water. Severe corrosion of heat exchanger, pipes, valves etc. can result where appreciable quantities of free carbon dioxide are present in water. Corrosion from carbon dioxide is boiler system is most frequently encountered in steam and return lines. Even though the boiler feed water contains no free carbon dioxide, appreciable concentration of this corrosive gas may come from carbonate and bicarbonate of the boiler feed water. Although Bangladesh Environment Conservation Act (1997) does not set any limit for its presence in drinking water, limits for carbon dioxide has been prescribed for many industrial uses.

THEORY

This test for determination of ***free carbon dioxide*** in water is based on the titration of the sample with standard sodium hydroxide solutions in the presence of phenolphthalein indicator (which changes color from colorless to pink at a pH of about 8.3). The carbon dioxide reacts with sodium hydroxide to form sodium bicarbonate with a consequent increase in pH value. The reaction of sodium hydroxide with free carbon dioxide reaches a completion at a pH of about 8.3. As soon as this pH is reached, a slight pink color is developed and this is taken as end point of reaction.

The reactions may be considered to occur as follows:

$$CO_2 + H_2O = H_2CO_3 \quad (1)$$
$$H_2CO_3 + NaOH = HCO_3^- + Na^+ + H_2O \quad (2)$$
$$HCO_3^- + NaOH = CO_3^{2-} + Na^+ + H_2O \quad (3)$$

REAGENTS

1. Phenolphthalein indicator
2. Standard N/44 sodium hydroxide

APPARATUS

1. Beaker : 2 pc.
2. Measuring cylinder : 1 pc.
3. Dropper : 1 Pc.
4. Stirrer : 1 pc.
5. Burette : 1 pc.

PROCEDURE

(1) Take 100 ml. of sample into a beaker and same quantity of distilled water into another. Add 10 drops of Phenolphthalein indicator to each. If a pink color develops, no carbon dioxide is present in the water sample. Otherwise, go to the next step.

(2) Add N/44 sodium hydroxide solution from a burette to the sample and stir gently until a slight permanent pink color appears as compared with the distilled water. Record the ml. of sodium hydroxide used.

CALCULATION

Carbon dioxide present (mg/L) = Multiplying Factor (MF) × milliliter of N/44 NaOH added

where, MF normality of NaOH × equivalent wt. of CO_2 × 1000 / (milliliter of sample taken)

Note: Since excess carbon dioxide, if present in water, easily escapes to the atmosphere, tests for carbon dioxide should be performed immediately after collection of water sample (especially for ground water samples which usually contain high carbon dioxide). If this is not possible, the sample bottle should be completely filled and stoppered and the sample should be kept at a temperature lower than that at which it was collected.

Where the free carbon dioxide of the water sample is high, there may be some loss of carbon dioxide to the atmosphere during the titration process. To check this, it is advisable to secure a second sample. Complete determination of carbon dioxide on the first sample. Then take the second sample (100 ml.) and immediately add the full amount of N/44 sodium hydroxide solution used in the titration of the first sample. Add 10 drops of phenolphthalein indicator and if the sample remains colorless, add additional N/44 sodium hydroxide to the end point (till the slight pink color appears) and accepting this second test as more accurate titration.

DETERMINATION OF ACIDITY

Phenolphthalein acidity (often called CO_2 acidity) is defined as the amount of standard base (usually N/50 NaOH) required to raise the pH of a sample of water to the phenolphthalein end point of 8.3.

Hence, acidity could be easily determined from the results of CO_2 determination as follows:

Phenolphthalein acidity as mg/L $CaCO_3$ = CO_2 (mg/L) × (50/ 44)

ASSIGNMENT QUESTIONS

(1) Discuss the environmental significance of carbon-dioxide in water

(2) Why groundwater usually contains more carbon-dioxide than surface water?

(3) Why the test for carbon-dioxide should be performed immediately after collection of the water sample? Explain.

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Experiment No. 6
Determination of Alkalinity of Water

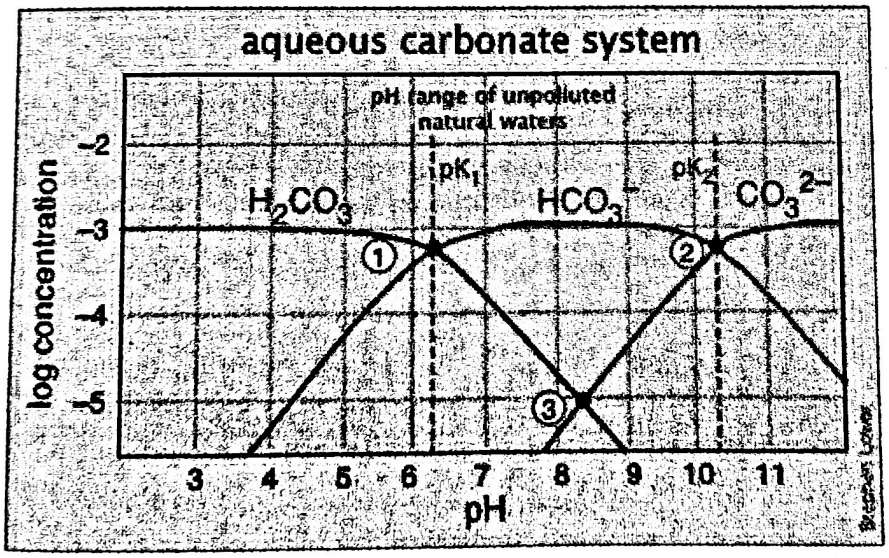
INTRODUCTION

The ***alkalinity*** of water is a measure of its capacity to neutralize acids. The term ***acid neutralizing capacity*** **(ANC)** is sometimes used. The alkalinity of natural waters is due primarily to the salts of weak acids, although weak or strong bases may also contribute. Bicarbonates represent the major form of alkalinity, since they are formed in considerable amounts from the action of carbon dioxide upon basic materials in the soil (as shown in Eq.1).

$$CO_2 + CaCO_3 + H_2O = Ca^{2+} + 2\,HCO_3^- \qquad (1)$$

Other salts of weak acids, such as borates, silicates, and phosphates, may be present in small amounts. A few organic acids, for example humic acid, form salts that add to the alkalinity of natural waters.

Although many materials may contribute to the alkalinity of water, the major portion of the alkalinity in natural waters is caused by three major types of chemical constituents: (i) hydroxide, (ii) carbonate, and (iii) bicarbonate. For most practical purposes, alkalinity due to other materials in natural waters is insignificant and may be ignored. The predominance of different carbonate species (closed syatem) is shown in Fig 1.



$pK_1 = 6.37$
$pK_2 = 10.33$

Figure 1: Logarithmic concentration diagram for 0.001 M Carbonic Acid (Source: Sawyer et al., 1994)

ENVIRONMENTAL SIGNIFICANCE

The alkalinity of waters is due principally to salts of weak acids and strong bases, and such substances act as buffer to resist a drop in pH resulting from acid additions. Alkalinity is thus a measure of the buffer capacity and in this sense is used to a great extent in wastewater treatment practice. Alkalinity is

very important in iron removal from water by oxidation-precipitation process. It has been found that rate of oxidation of dissolved ferrous iron (Fe^{2+}) into insoluble ferric iron is vary slow for low alkaline waters (alkalinity less than 130 mg/l as $CaCO_3$). Because the alkalinity of many waters is primarily a function of carbonate, bicarbonate and hydroxide content, it is taken as an indication of the concentration of these constituents. Alkalinity of water has little public health significance. Highly alkaline waters are usually unpalatable.

THEORY

Alkalinity is measured volumetrically by titration with *N/50* (i.e., 0.02 N) H_2SO_4 and is reported in terms of equivalent $CaCO_3$. For samples with initial pH is above 8.3, the titration is made in two steps. In the first step, the titration is conducted until the pH is lowered to 8.3, the point at which phenolphthalein indicator turns from pink to colorless. The second phase of the titration is conducted until the pH is lowered to about *4.5*, corresponding to the methyl orange (or bromocresol green) end point. When the pH of a sample is less than 8.3, a single titration is made to a pH of *4.5*. The first end point of pH 8.3 corresponds to the equivalence point for the conversion of carbonate to bicarbonate (Eq. 2, Fig 1). When alkalinity is measured to the phenolphthalein end point (pH 8.3), it is called phenolphthalein alkalinity which is due to the presence of either hydroxide or carbonate or both.

$$CO_3 + H^+ = HCO_3^- \qquad (2)$$

The pH of about 4.5 used as the end point for the second step of the titration corresponds approximately to the equivalence point for the conversion of bicarbonate ion to carbonic acid (Eq. 3, Fig 1). When alkalinity is measured to the methyl orange (or bromocresol green) end point, it is called methyl orange alkalinity or total alkalinity which is due to the presence of hydroxide, carbonate, and bicarbonate.

$$HCO_3^- + H^+ = CO_3^{2-} \qquad (3)$$

REAGENTS

1. Phenolphthalein indicator
2. Standard 0.02N (or N/50) sulfuric acid
3. Methyl orange indicator

APPARATUS

1.Beaker	:2 pc
2. Measuring cylinder	:lpc
3. Dropper	:lpc
4. Stirrer	:lpc

PROCEDURE

(I) Take 100 ml. of the sample into one beaker and the same amount of distilled water into another beaker.

(2) Measure pH of the sample with a pH meter.

(3) Add 3 drops of phenolphthalein indicator to each. If the sample becomes pink, go to step (3). Otherwise, go to step (4).

(4) Add 0.02N H_2SO_4 acid from a burette until the pink color just disappears. Record the ml. of the acid used.

(5) Add 3 drops of methyl orange indicator to each beaker. If the sample becomes yellow add 0.02N H_2SO_4 acid until the first change in color is noted. The end point is a slight orange tinge. Record the ml. of acid used.

CALCULATION

Thus, Phenolphthalein Alkalinity = (ml of N/50 H_2SO_4 to pH 8.3) × 1000 / (ml of sample)

= (ml of H_2SO_4 to pH 8.3) × Multiplying Factor (MF) (4)

and, Total Alkalinity = (total ml of N/50 H_2SO_4 to pH approx. 4.5) × 1000 / (ml of sample)
= (total ml of H_2SO_4 to pH approx. 4.5) × Multiplying Factor (MF) (5)

where, MF = normality of H_2SO_4 × equivalent wt. of $CaCO_3$ × 1000/ (ml of sample taken)

HYDROXIDE, CARBONATE, AND BICARBONATE ALKALINITY

In water analysis it is often desirable to know the kinds and amounts of the various forms of alkalinity present. This information is especially needed in water-softening processes and in boiler-water analysis.

There are three procedures commonly used to make these calculations:
(1) Calculation from alkalinity measurements alone,
(2) Calculation from alkalinity plus pH measurements, and
(3) Calculation from equilibrium equations.

The first is the classical method and is based on empirical relationships for the calculation of the various forms of alkalinity from phenolphthalein and total alkalinities. This is intended for use by technicians and others who do not have knowledge on the fundamental chemistry involved.

The second procedure gives sufficiently accurate estimates for most practical purposes, and also makes use of phenolphthalein and total alkalinity measurements. In addition, an accurate initial pH measurement is required for the direct calculation of hydroxide alkalinity.

In the third procedure, the various equilibrium equations for carbonic acid are used to compute the concentrations of the various alkalinity forms. This method gives reasonably accurate results for constituents, even when present in fractional mg/l range. A total alkalinity, as well as a pH measurement, is required. In addition, a dissolved solid measurement to correct for 'ion activity and a temperature measurement for the selection of a proper equilibrium constant are needed. However, reasonably accurate results are obtained without these two data if the dissolved solids concentration is not too high and water temperature is around 25 ^{0}C. Description of all three procedures are available in Sawyer et al. (1994). The following section describes only the third procedure to determine hydroxide, carbonate and bicarbonate alkalinities.

Calculation from Equilibrium Equations
The relevant equilibrium equations are as follows:

$$H_2O = H^+ + OH^- \quad (6a)$$

$$K_W = \{H^+\}\{OH^-\} = 10^{-14} \text{ (at 25 °C)} \quad (6b)$$

$$H_2CO_3 = H^+ + HCO_3^- \quad (7a)$$

$$K_{A1} = \{H^+\}\{HCO_3^-\} / \{H_2CO_3\} = 10^{-6.3} \text{ (at 25 °C)} \quad (7b)$$

$$HCO_3^- = H^+ + CO_3^{2-} \quad (8a)$$

$$K_{A2} = \{H^+\}\{CO_3^{2-}\} / \{HCO_3^-\} = 10^{-10.3} \text{ (at 25 °C)} \quad (8b)$$

The alkalinity of water can be expressed as follows:

$$\text{Total alkalinity (mg/l as } CaCO_3) / 50{,}000 = (HCO_3^-) + 2\,(CO_3^{2-}) + (OH^+) - (H^+) \quad (9)$$

From the above equations, the following expressions for different alkalinities can be derived:

$$\text{Carbonate alkalinity (mg/l as } CaCO_3) = [50{,}000\ \{(\text{alkalinity}/50{,}000) + (H^+) - (K_w/(H^+)\}] / [1 + \{(H^+)/2K_{A2}\}] \quad (10)$$

$$\text{Bicarbonate alkalinity (mg/l as } CaCO_3) = [50{,}000\ \{(\text{alkalinity}/50{,}000) + (H^+) - (K_w/(H^+)\}] / [1 + \{2K_{A2}/(H^+)\}] \quad (11)$$

It should be noted that the equilibrium constants vary with temperature and activities of ions vary with concentration of ions in water. If temperature of water varies significantly from 25 ^oC and ionic strength of water is high (that is high concentration dissolved ions), appropriate corrections need to be incorporated in the above equations. Otherwise these equations would give reasonably accurate results. Sometimes actual concentrations of carbonate and bicarbonate ions may be desired. Conversions to mg/l of CO_3^{2-} and HCO_3^- are as follows:

$$\text{mg/l } CO_3^{2-} = \text{mg/l carbonate alkalinity} \times 0.6 \quad (12)$$

$$\text{mg/l } HCO_3^- = \text{mg/l bicarbonate alkalinity} \times 1.22 \quad (13)$$

Molar concentrations may be obtained as follows:

$$(CO_3^{2-}) = (\text{mg/l } CO_3^{2-}) / 60{,}000 \quad (14)$$

$$(HCO_3^-) = (\text{mg/l } HCO_3^-) / 61{,}000 \quad (15)$$

ASSIGNMENT QUESTIONS

(1) Discuss the environmental significance of alkalinity.

(2) Define total alkalinity, phenolphthalein alkalinity, and methyl orange alkalinity?

(3) Calculate hydroxide, bicarbonate, and carbonate alkalinities of a water sample with a total alkalinity of 250 mg/l as $CaCO_3$ and a pH of 7.8. Which type of alkalinity dominates the total alkalinity? Also calculate the concentrations of carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) ions. [Hint: Use Eqs. 10-15]

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Experiment No. 7
Determination of Hardness of Water

BACKGROUND

Hard waters are generally considered to be those waters that require considerable amounts of soap to produce a foam or lather and that also produce scale in hot-water pipes, heaters, boilers, and other units in which the temperature of water is increased substantially. The hardness of water varies considerably from place to place. In general, surface water is softer than groundwater. The hardness of water reflects the nature of the geological formations with which it has been in contact.

Hardness is caused by multivalent metallic cations. Such cations are capable of reacting with soap to form precipitates and with certain anions present in water to form scale. The principal hardness causing cations are the divalent calcium, magnesium, strontium, ferrous iron, and manganous ions. These cations and the important anions with they are associated are shown in Table 1 in the order of their relative abundance in natural waters. Aluminum and ferric ions are sometimes considered as contributing to the hardness of water. However, their solubility is so limited at pH values of natural waters that ionic concentrations are negligible. The hardness of water is derived largely from contact with the soil and rock formations.

Table 1. Principal cations causing hardness and the major anions associated with them

Cations causing Hardness	Anion
Ca^{2+}	HCO_3^-
Mg^{2+}	SO_4^{2-}
Sr^{2+}	Cl^-
Fe^{2+}	NO_3^-
Mn^{2+}	SiO_3^{2-}

ENVIRONMENTAL SIGNIFICANCE

Hard waters are as satisfactory for human consumption as soft waters. Because of their adverse action with soap, however, their use for cleansing purpose is quite unsatisfactory, unless soap costs are disregarded. Soap consumption by hard waters represents an economic loss to the water user. Sodium soaps react with multivalent metallic cations to form a precipitate, thereby losing their surfactant properties. In recent years these problems have been largely alleviated by the development of soaps and detergents that do not react with hardness.

Boiler scale, the result of the carbonate hardness precipitation, may cause considerable economic loss through fouling of water heater and hot water pipes. Changes in pH in the water distribution systems may also result in deposits of precipitates. Bicarbonates begin to convert to the less soluble carbonates at pH values above 9.0.

Magnesium hardness, particularly associated with the sulfate ion has a laxative effect on persons unaccustomed to it. Magnesium concentrations of less than 50 mg/l are desirable in potable waters, although many public water supplies exceed the amount. Calcium hardness presents no public health problem. In fact, hard water is apparently beneficial to the human cardiovascular system.

Water are generally classified in terms of the degree of hardness as follows:

Soft	<50 mg/l as $CaCO_3$
Moderately hard	50 - 150 mg/l as $CaCO_3$
Hard	150 - 300 mg/l as $CaCO_3$
Very hard	> 300 mg/l as $CaCO_3$

THEORY

Hardness is usually expressed in terms of $CaCO_3$. Perhaps the most accurate method of determining hardness is by a calculation based upon the divalent ions found through complete cation analysis. This method is preferred where complete analyses are available. Hardness caused by each cation can be calculated as follows:

$$\text{Hardness (as mg/l } CaCO_3) = M^{2+} \text{ (mg/l)} \times 50 / (\text{Equivalent weight of } M^{2+}) \quad (1)$$

where, M^{2+} = concentration of divalent metal cation in mg/l and 50 is the equivalent weight of $CaCO_3$.

However, complete analyses of metal ions are not always available and in laboratory total hardness is usually determined by the ***EDTA Titrimetric Method***. This method yields very precise and accurate results and is the method of choice in most laboratories.

The EDTA Titrimetric method involves the use of solutions of (ethylene-diamine-tetra acetic acid) (EDTA) or its sodium salt as the titrating agent. These compounds are chelating agents and form extremely stable complexes with Ca^{2+}, Mg^{2+} and other divalent cations causing hardness, as shown in Eq. 2.

$$M^{2+} + EDTA = [M.EDTA]_{complex} \quad (2)$$

The successful use of EDTA for determining hardness depends upon having an indicator present to show when EDTA is present in excess, or when all the ions causing hardness have been complexed.

The Eriochrome Black T dye serves as an excellent indicator to show when all the hardness ions have been complexed. When a small amount of Eriochrome Black T, having a blue color, is added to hard water with a pH of about 10.0, it combines with a few of the Ca^{2+} and Mg^{2+} ions to form a weak complex ion which is wine red in colour (Eq. 3).

$$M^{2+} + \text{Eriochrome Black T} = (\text{M.Eriochrome Black T})_{complex} \quad (3)$$
$$\text{(wine red colour)}$$

During titration with EDTA, all free hardness ions are complexed according to Eq. 2. Finally, the EDTA disrupts the wine red (M.Eriochrome Black T) because it is capable of forming more stable complex with the hardness ions. This action frees the Eriochrome Black T indicator, and the wine red color changes to a distinct blue colour (Eq. 4), indicating the end of titration.

(M.Eriochrome Black T) $_{complex}$ + EDTA(M.EDTA)conipiex + Eriochrome Black T (4)
(wine red color) (blue color)

REAGENTS:

(1) Buffer solution (for attaining a pH close to 10.0).
(2) Eriochrome Black T dye.
(3) Standard EDTA solution.

(Note: The above three reagents have been procured from HACT Co., USA)

APPARATUS

1. Beaker	: 1 pc
2. Measuring cylinder	: 1 pc
3. Dropper	: 1 pc
4. Srirrer	: 1 pc
5. HACH auto-titration device	: 1 set

PROCEDURE

(1) Take 50 ml sample in a 150 ml beaker.
(2) Add one ml of standard buffer solution (supplied by HACH) to raise the pH of water sample to about 10.0 (Note: If 100 ml sample is taken, add 2 ml buffer).
(3) Add one packet of Eriochrome Black I dye (supplied by HACH) indicator to the beaker. The sample would turn wine-red (if hardness is present).
(4) Fit the cartridge containing standard EDTA solution to the titrator device (supplied by HACH).
(5) Turn the flow control knob of the device until the solution starts to come out of the tube fitted to the cartridge. Take initial reading of the counter.
(6) Immerse the tube fitted to the cartridge into the water sample and start titrating (under constant stirring) by turning the flow control knob of the auto-titrator. Continue until the wine-red color of the sample changes to blue. Take final reading of the counter.

CALCULATION

Total hardness as mg/l $CaCO_3$ = 2 × (initial counter reading - final counter reading)

If 100 ml sample is taken, hardness = (initial counter reading - final counter reading)

TYPES OF HARDNESS

In addition to total hardness, it is often desirable and sometimes necessary to know the type of hardness present. Hardness is classified in two ways: (1) with respect to the metallic ion (calcium and magnesium

hardness), and (2) with respect to the anions associated with the metallic ions (carbonate and non-carbonate hardness).

Calcium and Magnesium Hardness

Calcium and magnesium cause by far the greatest portion of the hardness occurring in natural waters. Sometimes, it is important to know the amounts of calcium and magnesium hardness in water. For example, it is necessary to know the magnesium hardness or the amount of Mg> in order to calculate lime requirement in lime-soda ash softening. The calcium and magnesium hardness may be calculated from the complete chemical analysis. If calcium hardness is determined, magnesium hardness is obtained by subtracting calcium hardness from total hardness as follows:

Total hardness - calcium hardness = magnesium hardness (5)

Carbonate and Non-carbonate Hardness

The part of total hardness that is chemically equivalent to the bicarbonate plus carbonate alkalinities present in water is considered to be ***carbonate hardness***. Since alkalinity and hardness are both expressed in terms of $CaCO_3$, the carbonate hardness can be found as follows:

When alkalinity < total hardness,
Carbonate hardness (mg/l) = alkalinity (mg/l) (6)

When alkalinity ≥ total hardness,
Carbonate hardness (mg/l) = total hardness (mg/l) (7)

Carbonate hardness is of particular interest because the bicarbonate and carbonate ions with which it is associated tend to precipitate this portion of the hardness at elevated temperatures such as occur in boilers or during the softening process with lime. Carbonate hardness was formerly called ***temporary hardness*** because it can be caused to precipitate by prolonged boiling (Eq. 8).

$$Ca^{2+} + 2HCO_3 = CaCO_3\,(s) + CO_2 + H_2O \quad (8)$$
$$Ca^{2+} + 2HCO_3 + Ca(OH)_2 = 2CaCO_3\,(s) + 2H_2O \quad (9)$$

The amount of hardness which is in excess of the carbonate hardness is called *noncarbonate hardness*, and can be estimated as follows:

Non-carbonate hardness (NCH) = total hardness - carbonate hardness (10)

Non-carbonate hardness was formerly called *permanent hardness* because it cannot be removed or precipitated by boiling. Non-carbonate hardness cations are associated with sulfate, chloride, and nitrate anions.

Pseudo-Hardness

Sea, brackish, and other waters that contain appreciable amounts of Na^+ interfere with the normal behavior of soap because of the common ion effect. Sodium is not a hardness-causing cation, and so this action which it exhibits when present in water in high concentration is termed *pseudo-hardness*.

ASSIGNMENT QUESTIONS

(1) What is hardness and how it is caused? Discuss the environmental significance of hardness.

(2) A water sample has the following analysis:

Na^+	20 mg/l	Cl^-	40 mg/l
Ca^{2+}	30 mg/l	HCO_3^-	67 mg/l
Mg^{2+}	10 mg/l	CO_3^{2-}	0 mg/l
Sr^{2+}	2 mg/l	SO_4^{2-}	5 mg/l
K^+	30 mg/l	NO^-	10 mg/l

What is the total hardness, carbonate hardness, and non-carbonate hardness in mg/l as $CaCO_3$?

(3) What do you understand by temporary hardness? A water sample has a total alkalinity of 265 mg/l and a total harness of 315 mg/l as $CaCO_3$. Calculate carbonate and non-carbonate hardness (in mg/l as $CaCO_3$) of the sample.

Course Number : CE 332
Course Name : Environmental Engineering Lab
Course Credit : 1.50

Experiment No. 8
Determination of Chloride Concentration of Water

INTRODUSTION

Chlorides occur in all natural waters in widely varying concentration. The chloride content normally increases as the mineral content increases. Upland and mountain supplies usually are quite low in chlorides, whereas river and groundwater usually have a considerable amount. Sea and ocean waters represent the residues resulting from partial evaporation of natural waters that flow into them and chloride levels are very high.

Chlorides gain access to natural waters in many ways. The solvent power of water dissolves chlorides from topsoil and deeper formations. Spray from the ocean is carried inland as droplets or as minute salt crystals, which result from evaporation of the water in the droplets. These sources constantly replenish the chlorides in inland areas where they fall. Ocean and sea waters invade the rivers that drain into them, particularly the deeper rivers. The salt water, being denser, flows upstream under the fresh water which is flowing downstream. There is a constant intermixing of the salt water with the fresh water above. Groundwater in areas adjacent to the ocean is in hydrostatic balance with seawater. Over-pumping of groundwater produces a difference in hydrostatic head in favor of the seawater, and it introduce into the fresh water area. Such intrusion has occurred in many areas of the coastal southern region of Bangladesh

Human excreta, particularly the urine, contain chloride in an amount about equal to the chlorides consumed with flood and water. This amount average about 6 gm of chlorides per person per day and increases the amount of Cl^- in municipal wastewater about 15 mg/l above that of the carriage water. Thus, wastewater effluents add considerable chlorides to receiving streams. Many industrial wastes (e.g., tannery waste) also contain appreciable amount of chlorides.

ENVIRONMENTAL IMPORTANCE

Chlorides in reasonable concentrations are not harmful to human. At concentrations above 250 mg/l they give a salty taste to water which is objectionable to many people. For this reason, chlorides are generally limited to 250 mg/l in supplies intended for public use. In many areas of the world where water supplies are scarce, source containing as much as 2,000 mg/l are used for domestic purposes without the development of adverse effects, once the human system becomes adapted to the water. According to Bangladesh Environment Preservation Act (1997), drinking water standard for chloride is 150-600 mg/l; but for coastal regions of Bangladesh, the limit has been relaxed to 1000 mg/l.

Before the development of bacteriological testing procedures, chemical testes for chloride and for nitrogen, in its various forms, served as the basis of detecting contamination of

groundwater by wastewater. Chlorides are used to some extent as tracers in sanitary engineering practice; however, they have been replaced to a great extent by organic dyes.

THEORY

Chlorides may be readily measured by means of volumetric procedures. For most purposes, the ***Mohr Method*** employing silver nitrate as the titrant and potassium chromate as the indicator is satisfactory. However, the procedure using mercuric nitrate and diphenylcarbazone as the indicator has certain inherent advantages over the *Mohr Method.*

In Mohr method the chloride of the sample in determined by titration with a standard silver nitrate solution in the presence of potassium chromate indicator. The "Standard Method" recommends a 0.0141 N silver nitrate solution which corresponds to a N/71 solution or a solution in which each milliliter is equivalent to 0.50 mg of chloride ion.

In the titration, chloride is precipitated as a white silver chloride (Eq. 1)

$$Ag^+ + Cl^- = AgCl \text{ (white precipitate)} \qquad (1)$$

The end point of titration cannot be detected visually unless an indicator capable of demonstrating the presence of excess Ag^+ is present. The indicator normally used in potassium chromate, which supplies chromate ions. As the concentration of Cl^- ions becomes exhausted, the silver ion concentration increases and a reddish brown precipitate of silver chromate is formed (Eq. 2).

$$2Ag^+ + CrO_4^{2-} = Ag_2CrO_4 \text{ (reddish brown precipitate)} \qquad (2)$$

This is taken as evidence that all chloride has been precipitated. Since an excess Ag^+ is needed to produce a visible amount of Ag_2CrO_4, the indicator error is subtracted from all titrations.

Several precautions must be observed in determination of chloride for ensuring accuracy:

(1) A uniform sample size must be used, preferably 100 ml (or 50 ml), so that ionic concentrations needed to indicate the end point will be constant.
(2) The pH must be in the range of 7 to 8 because Ag^+ is precipitated as AgOH at high pH levels and then CrO_4^{2-} is converted to $Cr_2O_7^{2-}$ at low pH levels.
(3) A definite amount of indicator must be used to provide a certain concentration of CrO_4^{2-}, otherwise Ag_2CrO_4 may form too soon or not soon enough.

The indicator error or blank varies somewhat with the ability of individuals to detect a noticeable color change. The usual range is 0.2 to 0.4 ml of titrant. An error of 0.2 ml will be used in this class.

REAGENTS

(1) Potassium chromate indicator
(2) Silver nitrate solution (0.0 141N)

APPARATUS

(1) Measuring cylinder (100 ml)	: 1 pc.
(2) Beaker	: 1 pc.
(3) Dropper	: 1 pc.
(4) Stirrer	: 1 pc.

PROCEDURE

(1) Take 50 ml. of the sample in a beaker and add 5 drops (about 1 ml) of potassium chromate indicator to it.
(2) Add standard (0.0141 N) silver nitrate solution to the sample from a burette, a few drops at a time, with constant stirring until the first permanent reddish color appears. This can be determined by comparison with distilled water blank. Record the ml. of silver nitrate used.
(3) If more than 7 or 8 ml. of silver nitrate solution are required, the entire procedure should be repeated using a smaller sample diluted to 50 ml with distilled water.

CALCULATION

Chloride, Cl^- (mg/l) = (ml. of $AgNO_3$ used -"error" or "blank") × Multiplying Factor (MF)

where, MF= normality of $AgNO_3$ × equivalent wt. of Cl^- × 1000 / (milliliter of sample taken)

ASSIGNMENT QUESTIONS

(1) Discuss the environmental significance of chloride in water.

(2) What precautions should be taken in the determination of chloride concentration by Mohr method? Explain why.

(3) In determination of chloride, why an indicator "blank" or "error" is subtracted from the amount of silver nitrate used in titration? Explain.

Course Number : CE 332
Course Name : Environmental Engineering Lab
Course Credit : 1.50

Experiment No. 9
Determination of Iron Concentration of Water

INTRODUCTION

Iron, as well as manganese, creates serious problems in public water supplies. The problems are most critical for groundwater. Iron exists in soils and minerals as insoluble ferric oxide/hydroxide and iron sulfide (pyrite). In some areas, it also occurs as ferrous carbonate which is very slightly soluble. Since groundwater usually contains significant amounts of carbon dioxide, appreciable amounts of ferrous carbonate may be dissolved according to Eq. 1.

$$FeCO_3 + CO_2 + H_2O = Fe^{2+} + 2HCO_3^- \quad (1)$$

If reducing (anaerobic) conditions exist in groundwater environment, the insoluble ferric iron [Fe^{3+}] is reduced to more soluble ferrous iron [Fe^{2+}] and iron concentration in water increases. There seems to be enough evidence to suggest that development of reducing (anaerobic) condition is essential for appreciable amount of iron (as well as manganese) to gain entrance into water.

ENVIRONMENTAL SIGNIFICANCE

As far as is known, humans suffer no harmful effects from drinking waters containing iron and manganese. Such waters, when exposed to the air so that oxygen can enter, become turbid and highly unacceptable from the aesthetic viewpoint owing to the oxidation of iron and manganese to the Fe^{3+}, and Mn^{4+} states that form colloidal precipitates. The rates of oxidation are not rapid, and thus reduced forms can persist for some time in aerated waters. This is especially true when the pH is below 6 with iron oxidation and below 9 with manganese oxidation. The rates may be increased by the presence of certain inorganic catalysts of through the action of micro-organisms.

Both iron and manganese interfere with laundering operations, impart objectionable stains to plumbing fixtures and cause difficulties in distribution systems by supporting growths of iron bacteria, iron also imparts a taste to water which is detectable at very low concentrations. According to Bangladesh Environment Preservation Act (1997), drinking water standard for iron is 0.3-1.0 mg/l.

THEORY

Iron may be present in two forms, namely the reduced form (ferrous, Fe^{2+}) and the fully oxidized form (ferric, Fe^{3+}). Ferric iron is seldom found in true solution in natural waters,

unless they are highly acidic, because of the formation of insoluble ferric hydroxides. Ferrous iron is more likely to be found in true solution, although it is easily oxidized to the ferric state and precipitated in alkaline waters as ferric hydroxide.

Ferric iron is determined by producing a red-colored iron compound, ferric thiocyanate, by the addition of potassium thiocyanate (Eq. 2).

$$Fe^{3+} + 3\ KCNS = Fe(CNS)_3 + 3\ K^+ \qquad (2)$$

The quantity of ferric iron is determined by comparison with the red color produced by standard iron solutions.

Since some iron may exist as iron hydroxide precipitates, it is necessary to make sure that all iron is in soluble condition. This is done by treating the sample to be tested with hydrochloric acid to dissolve the ferric hydroxide. For determination of total iron following this procedure, it must be ensured that all iron exist in ferric form (Fe^{3+}). This is most readily accomplished by using potassium permanganate, an oxidizing agent.

Ferrous iron is also determined colorimetrically by producing a blue compound, known as Turnbull's blue, by the addition of potassium ferriocyanate (Eq. 3).

$$3\ Fe^{2+} + 2\ K_3Fe(CN)_6 = Fe_3\ [Fe(CN)_6]_2 + 6\ K^+ \qquad (3)$$

The quantity of ferrous is determined by comparison with the color produced by standard iron solutions. Standard iron solutions (in both cases) are prepared from ferrous ammonium sulfate crystals [$FeSO_4(NH_4)_2SO_4.6H_2O$].

REAGENTS

(1) Hydrochloric acid
(2) Potassium permanganate solution
(3) Potassium thiocyanate solution
(4) Standard iron solution

APPARATUS

(1) Nessler tube (2)
(2) Measuring cylinder (1)
(3) Dropper (1)

PROCEDURE

(1) Place 100 ml. of the water sample in a Nessler tube.
(2) Add 5 ml. of dilute hydrochloric acid.
(3) Add two drops of potassium permanganate solution.

(4) Add 5 ml. of potassium thiocyanate solution. The solution would turn brown if iron is present.

(5) Compare the brown color formed with the standard prepared as follows:
   (a) Add 100 ml. of distilled water in a Nessler tube.
   (b) Add 5 ml. of the dilute hydrochloric acid.
   (c) Add two drops of potassium permanganate solution.
   (d) Add 5 ml. of potassium thiocyanate solution.
   (e) Add 0.2 ml. at a time of the standard iron solution until the color of the standard and the sample match.

CALCULATION

Iron present (mg/l) = ml. of the standard iron solution used.

ASSIGNMENT QUESTIONS

(1) Discuss the environmental significance of "iron" in water.

(2) Why hydrochloric acid and potassium permanganate are added to the sample in the determination of iron.

(3) Groundwater with high dissolved iron concentration often becomes turbid after extraction (in contact with air). Explain why.

Course Number : CE 332
Course Name : Environmental Engineering Lab
Course Credit : 1.50

Experiment No. 10
Determination of Biochemical Oxygen Demand (BOD)

INTRODUCTION

When biodegradable organic matter/waste (the most common category of pollutant affecting surface water) is discharged into a water body, micro-organisms (especially bacteria), fed on the wastes, breaking it down into simpler organic and inorganic substances. When this decomposition takes place in an aerobic environment (i.e. in presence of oxygen; Eq. 1), the process is termed as "Aerobic Decomposition", which produce non-objectionable stable products (e.g., CO_2, SO_4, PO_4, NO_3) and the process draws down the dissolved oxygen (DO) content of water.

$$\text{Organic matter} + O_2 \xrightarrow{\textit{Aerobic bacteria}} CO_2 + H_2O + \text{New cells} + \text{Stable products} \qquad (1)$$

When insufficient oxygen is available or when oxygen is exhausted by the aerobic decomposition of wastes, different set of microorganisms carry out the decomposition anaerobically (Eq. 2) producing highly objectionable products including H_2S, NH_3 and CH_4.

$$\text{Organic matter} \xrightarrow{\textit{Anaerobic bacteria}} CO_2 + CH_4 + \text{New cells} + \text{Unstable products} \qquad (2)$$

The amount of oxygen required by microorganisms to oxidize organic wastes aerobically called "Biochemical Oxygen Demand" (BOD). BOD may have various units, but most often it is expressed in mg of oxygen required per liter of water/ wastewater (mg/L). The total amount of oxygen that will be required for bio-degradation is an important measure of the impact that a given stream would have on the receiving water body.

Dissolved oxygen is the most commonly used indicator of the general health of a surface water body. If DO goes below 4 to 5 mg/L (due to decomposition of organic wastes), forms of life that can survive begin to be reduced. When anaerobic condition exists, higher life forms are killed or driven off. Noxious (unhealthy) condition, including floating sludge, bubbling, odorous gases, and slimy fungus growth prevails.

THEORY

The biochemical oxygen demand (BOD) determination is an empirical test, in which standardized procedures are used to determine the oxygen requirements of wastewater, effluents and polluted waters. It has become a standard practice to simply measure and report the oxygen

demand over a 5-day period, realizing that the ultimate oxygen demand (for complete degradation of organic matter) is considerably higher (and would take a much longer time to determine in the laboratory).

The 5-day BOD or BOD_5 is the amount of oxygen consumed by micro-organisms during the first 5-days of degradation. In its simplest form, BOD_5 test would involve putting a sample of water/ wastewater into a stoppered bottle, measuring the dissolved oxygen (DO) of the sample at the beginning of the test and again at the end of five days. The difference in DO would be the BOD_5 of the water/wastewater. Light must be kept out of the bottle to keep algae from adding oxygen by photosynthesis and the stopper is used to keep air from replenishing DO from outside.

To standardize the procedure, the test is run at a fixed temperature of 20°C. Since the oxygen demand of typical is several hundred mg/L and since the saturated value of DO of water at 20°C is only 9.1 mg/L, it is usually necessary to dilute the sample to keep the final DO above zero. If during the five days of experiment, DO drops to zero, then the test is invalid since more oxygen would have been removed had more been available.

The five-day BOD of a diluted sample is given by

$$BOD_5 = [DO_i - DO_f] \times DF \qquad (3)$$

where, DF = Dilution Factor = (Vol. of wastewater + dilution water)/ (Vol. of wastewater)

In some cases, it becomes necessary to seed the dilution water with microorganisms to ensure that there is an adequate bacterial population to carry out the biodegradation. In such cases, two sets of BOD bottles must be prepared, one for just the seeded dilution water (called the 'blank') and the other for the mixture of wastewater and dilution water. The change in DO for both is measured. The oxygen demand of the wastewater (BOD_w) is then determined from the following relationship:

$$BOD_m V_m = BOD_w V_w + BOD_d V_d \qquad (4)$$

where,
BOD_m = BOD of the mixture of wastewater and dilution water
BOD_d = BOD of the dilution water alone
V_w = Volume of wastewater
V_d = Volume of dilution water
V_m = Volume of the mixture = $V_w + V_d$

Dilution water and seed:

A wide variety of dilution waters have been used for BOD work. Natural surface waters have been used, but they suffer from a number of disadvantages including variable BOD, variable micro-organism population and variable mineral content. Tap water has also been used, but it also suffers from many of the limitations of the surface water plus the possibility of toxicity from chlorine residuals.
Synthetic dilution water prepared by adding different salts to distilled water has been found to be the most ideal for use as dilution water. The pH of the dilution water should be between 6.5 to

demand over a 5-day period, realizing that the ultimate oxygen demand (for complete degradation of organic matter) is considerably higher (and would take a much longer time to determine in the laboratory).

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$$BOD_m V_m = BOD_w V_w + BOD_d V_d \qquad (4)$$

where,
BOD_m = BOD of the mixture of wastewater and dilution water
BOD_d = BOD of the dilution water alone
V_w = Volume of wastewater
V_d = Volume of dilution water
V_m = Volume of the mixture = $V_w + V_d$

Dilution water and seed:

A wide variety of dilution waters have been used for BOD work. Natural surface waters have been used, but they suffer from a number of disadvantages including variable BOD, variable micro-organism population and variable mineral content. Tap water has also been used, but it also suffers from many of the limitations of the surface water plus the possibility of toxicity from chlorine residuals.
Synthetic dilution water prepared by adding different salts to distilled water has been found to be the most ideal for use as dilution water. The pH of the dilution water should be between 6.5 to

8.5. K, Na and Mg salts are added to distilled water to give buffering capacity and proper osmotic pressure. Ferric chloride, magnesium sulfate and ammonium chloride are added to supply the requirements for iron, sulfur and nitrogen. The phosphate buffer provides phosphorus that may be needed. The dilution water is usually well aerated to raise its DO level before mixing with wastewater.

A wide variety of materials have been used for "seeding" purpose. Experience has shown that domestic wastewater, particularly from combined sewer systems, provide a well-balanced population of mixed micro-organisms. Usually 2 ml of wastewater per liter of dilutes wastewater is sufficient. Some river waters are also satisfactory, but care must be taken to avoid using waters containing algae or nitrifying bacteria in significant amount. The dilution water should always be "seeded" to ensure a uniform population of micro-organisms.

Modeling of BOD:

It is often assumed that the rate of decomposition of organic waste is proportional to the amount of waste available. If L_t represents the amount of oxygen demand left after time t, then, assuming a first order reaction, we can write,

$$\frac{dL_t}{L_t} = -kL_t \tag{5}$$

where, k = BOD reaction rate constant ($time^{-1}$)

Integrating Eq.5, we get, $L_t = L_0 e^{-kt}$ (6)

where, L_0 = ultimate carbonaceous oxygen demand, which is the total amount of oxygen required by micro-organisms to oxidize the carbonaceous portion of the waste.

Now, $L_0 = BOD_t + L_t'$ (7)

And combining Eq. (6) and Eq. (7), we get

$$BOD_t = L_0 (1-e^{-kt}) \tag{8}$$

Figure 1(a) and 1(b) show the graphical representations of Eq. (6) and Eq. (8) respectively.

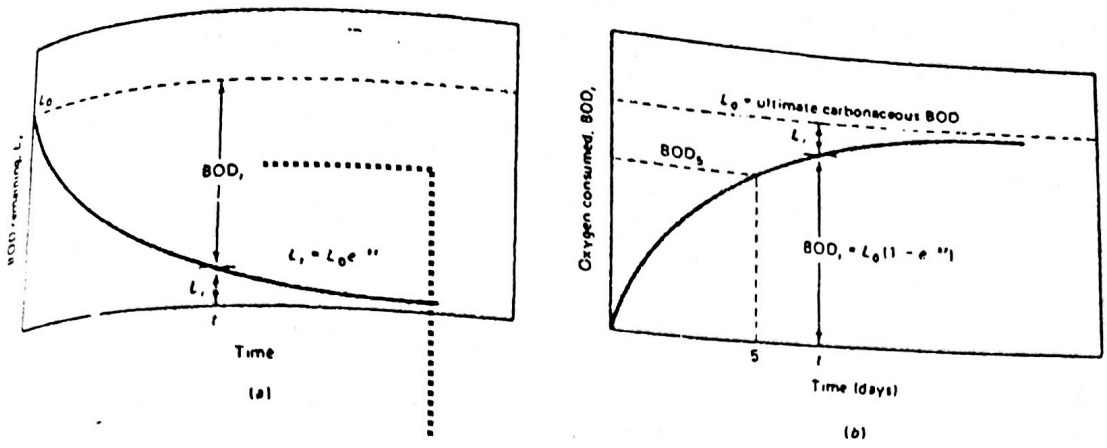


Figure 1. Idealized carbonaceous oxygen demand: (a) the BOD remaining as a function of time, and (b) the oxygen consumed or the biochemical oxygen demand as a function of time.

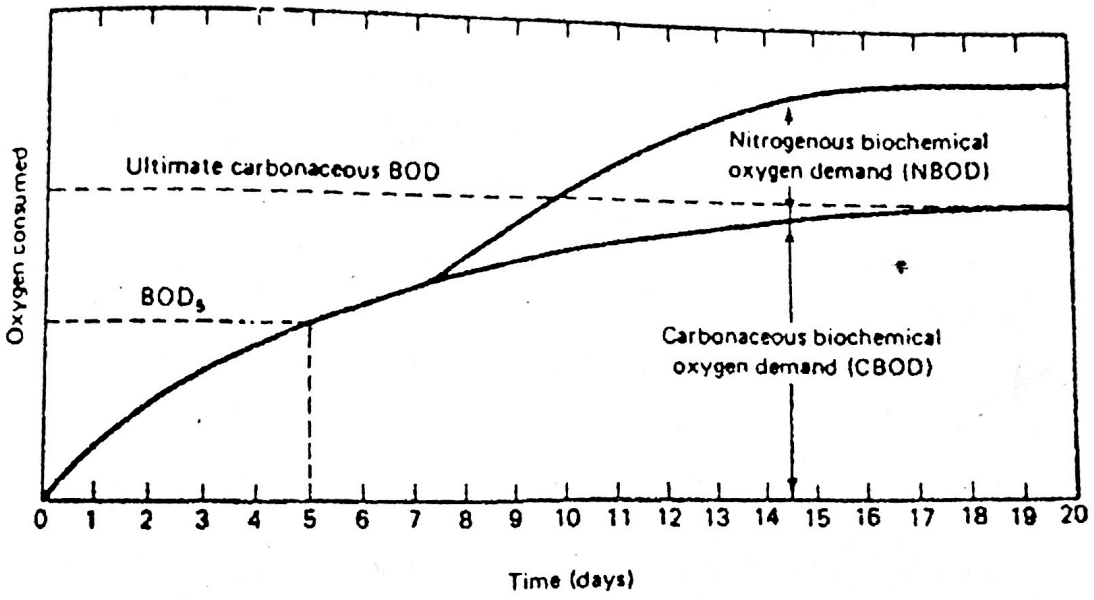


Figure 2. Illustration of Carbonaceous and Nitrogenous Biochemical Oxygen Demand

BOD reaction rate constant, k

The BOD reaction rate constant, k, is a factor that indicates the rate of biodegradation of wastes. As k increases, the rate at which dissolved oxygen is used increases, although the ultimate amount required, L_o, does not change.

$$K = k_{20}\,\theta^{(T-20)}$$

where, k_{20} is the reaction rate constant at standard 20°C.

The most commonly used value for θ is 1.047.

Beside the oxidation of carbonaceous organic matter, a significant additional demand may be caused by the oxidation of nitrogenous compounds. To distinguish this nitrogenous biochemical oxygen demand from the carbonaceous biochemical oxygen demand, the two are sometimes referred as NBOD and CBOD respectively. Figure 2 illustrates the two demands for a typical

municipal waste. Note that the NBOD does not begin to exert itself for at least 5 to 8 days. Therefore, most 5-day BOD tests are not affected by the nitrification process.

Theory of DO Determination:

The reactions involved in the various steps of the **Winkler Method** of dissolved oxygen (DO) determination are presented below:

Manganous sulfate reacts with potassium hydroxide in the alkaline potassium iodide solution to produce a white precipitate of manganous hydroxide.

$$MnSO_4 + 2KOH = \underset{\text{(white precipitate)}}{Mn(OH)_2} + K_2SO_4 \quad (9)$$

If the white precipitate is obtained, there was no dissolved oxygen in the sample and there is no need to proceed further. A brown precipitate shows that oxygen is present and reacted with the manganous hydroxide. The brown precipitate is manganic basic oxide and formed as follows:

$$2Mn(OH)_2 + O_2 = \underset{\text{(brown precipitate)}}{2MnO(OH)_2} \quad (10)$$

Upon the addition of (sulphuric) acid, this precipitate is dissolved, forming manganic sulfate:

$$MnO(OH)_2 + 2H_2SO_4 = Mn(SO_4)_2 + 3H_2O \quad (11)$$

This compound immediately reacts with potassium iodide, liberating iodine and resulting in the typical iodine (blue) coloration of the water.

$$Mn(SO_4) + 2KI = MnSO_4 + K_2SO_4 + \underset{\text{(blue)}}{I_2} \quad (12)$$

The quantity of iodine liberated by these reactions is equivalent to the quantity of oxygen present in the sample. The quantity of iodine is determined by titrating a portion of the solution with a standard solution of sodium thiosulfate solution.

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI \quad (13$$

REAGENTS

1. Manganous sulfate solution
2. Alkaline potassium iodide solution
3. 0.025N sodium thiosulfate
4. Starch solution (indicator)
5. Concentrated sulfuric acid.

APPARATUS

1. BOD bottle	: 3 nos.
2. Beaker (250 ml)	: 1 no.
3. Measuring cylinder	: 1 no.
4. Dropper	: 1 no.
5. Stirrer	: 1 no.

PROCEDURE (for determination of DO)

Fill two BOD bottles with sample (or diluted sample); the bottles should be completely filled. Determine initial DO (DO_i) in one bottle immediately after filling with sample (or diluted sample). Keep the other bottle in dark at 20°C and after particular days (usually 5-days) determine DO (DO_f) in the sample (or diluted sample). Dissolved oxygen (DO) is determined according to the following procedure:

(1) Add 1 ml of manganous sulfate solution to the BOD bottle by means of pipette, dipping in end of the pipette just below the surface of the water.

(2) Add 1 ml of alkaline potassium iodide solution to the BOD bottle in a similar manner.

(3) Insert the stopper and mix by inverting the bottle several times.

(4) Allow the "precipitates" to settle halfway and mix again.

(5) Again allow the "precipitates" to settle halfway.

(6) Add 1 ml. of concentrated sulfuric acid. Immediately insert the stopper and mix as before.

(7) Allow the solution to stand at least 5 minutes.

(8) Withdraw 100 ml of solution into an Erlenmeyer flask and immediately add 0.025N sodium thiosulfate drop by drop from a burette until the yellow color almost disappears.

(9) Add about 1 ml. of starch solution and continue the addition of the thiosulfate solution until the blue color just disappears. Record the ml. of thiosulfate solution used (disregard any return of the blue color).

CALCULATION

Dissolved Oxygen, DO (mg/L) = mL of 0.025N sodium thiosulfate added x 2

Calculate BOD of the sample according to Eq. 3 or Eq. 4.

ASSIGNMENT QUESTIONS

(1) A sample of sewage is mixed with water (no seeding done) in the ratio of 1:20 (i.e., 1 mL of sewage diluted to 20 mL by adding water) for BOD test. The initial DO is 8.5 mg/L and final DO, after 5 days, is 3.1 mg/L. Calculate BOD_5 of the sewage.

(2) A test bottle containing just seeded dilution water has its DO level drop by 0.8 mg/L in a 5-day test. A 300 ml BOD bottle filled with 30 ml of wastewater and the rest with seeded dilution water experiences a drop of 7.3 mg/L in the same period (5-day). Calculate the BOD_5 of the wastewater.

(3) In a BOD test on a diluted wastewater sample (1:20 dilution, but not seeded), the initial DO is 8.4 mg/L and final DO after 5 days is 4.2 mg/L. If the reaction rate constant is 0.22/day, calculate: (a) 5-day BOD of the wastewater, (b) ultimate carbonaceous BOD of the wastewater, (c) remaining oxygen demand after 5-days.

Course Number : CE 332
Course Name : Environmental Engineering Lab
Course Credit : 1.50

Experiment No.11
Determination of Chemical Oxygen Demand (COD)

INTRODUCTION

The chemical oxygen demand (COD) test is widely used as a means of measuring the organic strength of domestic and industrial wastes. This test allows measurement of a waste in terms of the total quantity of oxygen required for oxidation to carbon dioxide and water. The test is based on the fact that all organic compounds, with a few exceptions, can be oxidized by the action of strong oxidizing agents under acid conditions.

During the determination of COD, organic matter is converted to carbon dioxide and water regardless of the biological assimilability of the substance. For example, glucose and lignin are both oxidized completely. As a result, COD values are greater than BOD values, especially when biologically resistant organic matter (e.g., lignin) is present.

One of the chief limitations of COD test is its inability to differentiate between biologically oxidizable (biodegradable) and biologically inert (non-biodegradable) organic matter. In addition, it does not provide any evidence of the rate at which the biologically active material would be stabilized under conditions that exist in nature.

The major advantage of COD test is the short time required for evaluation. The determination can be made in about 3 hours rather than the 5-days required for the measurement of BOD. For this reason, it is used as a substitute for the BOD test in many instances.

THEORY

Potassium dichromate or potassium permanganate is usually used as the oxidizing agent in the determination of COD. In this class potassium permanganate would be used in the determination of COD. Potassium permanganate is selective in the reaction and attacks the carbonaceous and not the nitrogenous matter.

In any method of measuring COD, an excess of oxidizing agent must be present to ensure that all organic matter is oxidized as completely as possible within the power of the reagent. This requires that a reasonable excess be present in all samples. It is necessary, therefore, to measure the excess in some manner so that the actual amount can be determined. For this purpose, a solution of a reducing agent (e.g., ammonium oxalate) is usually used.

REAGENTS

1. Dilute sulfuric acid
2. Standard potassium permanganate
3. Standard Ammonium Oxalate

APPARATUS

1. Beaker (250 ml) :1 no.
2. Dropper 1 no.
3. Stirrer I no.

PROCEDURE (For water)

(1) Pipette 100 ml of the sample into a 250 ml Erlenmeyer flask.
(2) Add 10 ml. of diluted sulfuric acid and 10 ml of standard potassium permanganate.
(3) Heat the flask in a boiling water bath for exactly 30 minutes, keeping the water in the bath above the level of the solution in the flask. The heating enhances the rate of oxidation reaction in the flask.
(4) If the solution becomes faintly colored, it means that most of the potassium permanganate has been utilized in the oxidation of organic matter. In such a case, repeat the above using a smaller sample diluted to 100 ml with distilled water.
(5) After 30 minutes in the water bath, add 10 ml of standard ammonium oxalate into the flask. This 10 ml ammonium oxalate, which is a reducing agent, is just equivalent to the 10 ml potassium permanganate (oxidizing agent) added earlier. The excess of reducing agent (ammonium oxalate) now remaining in the flask is just equivalent to the amount of the oxidizing agent (potassium permanganate) used in the oxidation of organic matter.
(6) The quantity of ammonium oxalate remaining in the flask is now determined by titration with standard potassium permanganate. Titrate the content of the flask, while hot, with standard potassium permanganate to the first pink coloration. Record the ml of potassium permanganate used.

CALCULATION

COD (mg/l) = [mL of $KMnO_4$ used in Step (6) × (100) / mL of sample used]

ASSIGNMENT QUESTIONS

(1) What are the principal advantages and disadvantages of the COD test over the BOD test.
(2) Explain why COD values are greater than BOD values.
(3) What would be inferred from the following analytical results regarding the relative ease of biodegradability of each waste?

Waste type	5-day BOD (mg/l)	COD (mg/l)
A	240	300
B	100	500
C	120	240

Course Number : CE 332
Course Name : Environmental Engineering Lab
Course Credit : 1.50

Experiment No. 12
Chemical Coagulation (Alum Coagulation)

INTRODUCTION

Surface water generally contains a wide variety of colloidal impurities that may cause the water to appear turbid and may impart color to the water. Colloidal particles that cause color and turbidity are difficult to separate from water because the particles will not settle by gravity and are so small that they pass through the pores of most common filtration media. In order to be removed, the individual colloids must aggregate and grow in size so that they can settle by gravity. Chemical agents are used to promote colloid aggregation by destroying the forces that stabilize colloidal particles. The process of destroying the stabilizing forces and causing aggregation of colloids is referred to as chemical coagulation. Coagulation involves reduction of electrical forces of repulsion and promotion of "chemical type" interaction between colloids, which eventually results in settling of the colloids and accomplishes removal of turbidity and color. At higher coagulant doses, "charge reversal" is possible which may result in re-suspension of the colloids. Hence optimum coagulant doses are determined through laboratory model tests where the water to be treated are subjected to a range of doses of a coagulant and the removal efficiencies are observed.

Many authors use the term *"coagulation"* to describe the process by which the charge on particles is destroyed, and the term *"flocculation"* to describe the aggregation of particles into larger units. The chemical used for this purpose is called are called *coagulants*. The most common *coagulants* used in water and wastewater treatment are aluminum and ferric salts such as *alum,ferric chloride* and *ferric sulfate*.

The common metal salt alum (aluminum sulfate) is a good coagulant for water containing appreciable organic matter. The chemical formula used for commercial alum is $Al_2(S0_4)_3 14H_20$. Once dissolved in water, aluminum forms hydroxo-complexes and solids [e.g., $Al(OH)_3(s)$, $Al(OH)^{2+}$, $Al(OH)_{2-}$, $Al(OH)_4$; Eqs. 1-5] and as a result pH of water is lowered, especially if alkalinity of water is low. Theoretically, each mg/l of alum consume approximately 0.50 mg/l (as $CaCO_3$) of alkalinity. For water with low alkalinity, this may result in significant reduction in pH that may interfere with formation of aluminum hydroxide flocs. If the alkalinity is insufficient, coagulant aids such as lime ($CaOH_2$), soda ash (Na_2CO_3), activated silica and polyelectrolytes are used to provide the necessary alkalinity. Iron coagulants operates over a wider pH range and are generally effective in removing turbidity and color from water. However, they are usually more costly.

$$Al_2(S0_4)_3.\ 14\ H_2O\ (alum) = 2Al^{3+} + 3\ SO_4^{2-} \quad (1)$$
$$Al^{3+} + 3\ H_2O = Al(OH)_3\ (s) + 3\ H^+ \quad (2)$$
$$Al^{3+} + H_2O = Al(OH)^{2+} + H^+ \quad (3)$$
$$Al^{3+} + H_2O = Al(OH)_2^{+} + 2H^+ \quad (4)$$
$$Al^{3+} + H_2O = Al(OH)_4^{-} + H^+ \quad (5)$$

Besides the removal of turbidity and color, coagulation with alum and ferric chloride or ferric sulfate is also widely used for removal of heavy metal ions (e.g., lead, arsenic) from water. In this process heavy metal ions are primarily removed by adsorption (and subsequent precipitation) onto coagulated floes of metal (either aluminum or iron) hydroxides. Coagulation with alum and ferric chloride/sulfate have been successfully used for removal of arsenic from water.

REAGENTS

(1) Standard Alum solution.
(2) Standard Arsenic solution (if As removal is to be demonstrated)

APPARATUS

(1) Coagulation (stirring) device
(2) pH meter
(3) Turbidity meter
(4) Glass beakers (1000 ml; 6 nos.)

PROCEDURE

(1) Determine pH and turbidity of the water to be treated. You may be instructed to determine color and arsenic concentration of the water to be treated (if removal efficiencies of these parameters are to be determined).

(2) Fill six 1000 ml beakers each with 500 ml water to be treated.

(3) Add required (as instructed by teacher) coagulant (standard alum solution) to each beaker.

(4) Mix the samples in the beaker with the help of the stirring device. Subject the samples to one minute of rapid mixing followed by 15 minutes of slow mixing (about 40 rpm).

(5) Allow the floes to settle down for about 15 minutes. Observe the characteristics of the floes and the settling rates.

(6) Collect the supernatant from each beaker and measure pH and turbidity of each. You may be instructed to measure color and arsenic concentration of the supernatant samples (if removal efficiencies of these parameters are to be determined).

(7) Plot pH versus alum dose in a graph paper and observe effect of alum dose on pH.

(8) Plot turbidity versus the coagulant (alum) dose in a graph paper. Determine optimum dose of alum from this plot.
(9) Arsenic and color removal efficiencies could also be determined if measurements of color and arsenic concentrations are carried out.

ASSIGNMENT QUESTIONS

(1) What do you understand by coagulation and flocculation? Which coagulants are most commonly used for water and wastewater treatment?

(2) Why addition of alum may result in a drop in pH value. Discuss the effect of alum dose on pH from your experimental results.

(3) What is the primary mechanism by which heavy metal ions are removed during coagulation?

Course Number : CE 332
Course Name : Environmental Engineering Lab
Course Credit : 1.50

Experiment No. 13
Residual Chlorine and Chlorine Demand: Break Point Chlorination

INTRODUCTION

Chlorination of public water supplies and polluted waters serves primarily to destroy or deactivate disease-producing microorganisms. The practice of disinfection with chlorine is widely practiced. Chlorination may produce some adverse effects including taste and odor problem. In recent years, chlorination has been found to produce trihalomethanes (THMs) and other organics of health concern (THMs are suspected human carcinogens). Thus, use of alternative disinfectants, such as chlorine dioxide and ozone that do not cause this particular problem, is increasing.

CHEMISTRY OF CHLORINATION

Disinfectant capabilities of chlorine depend on its chemical form in water, which in turn is dependent on pH, temperature, organic content of water, and other water quality factors. Chlorine is used in the form of free chlorine [e.g., chlorine gas] or as hypochlorites [e.g., NaOC1 and $Ca(OCl)_2$]. Chlorine applied to water either as free chlorine or hypochlorite initially undergoes hydrolysis to form free chlorine consisting of aqueous molecular chlorine, hypochlorous acid and hypochlorite ion.

$$Cl_2 + H_2O = HOC1 + H^+ + Cl^- \quad (1)$$
$$NaOC1 = OC1^- + Na^+ \quad (2)$$
$$Ca(OC1)_2 = 2\,OC1^- + Ca^{2+} \quad (3)$$
$$HOC1 = H^+ + OCl^- \quad (4)$$

The relative proportion of these "free chlorine" forms is pH and temperature-dependent. Figure 1 shows a distribution diagram for the various chlorine species (Cl_2, HOC1, OCl^-) over a broad pH range.

Figure 1 shows that Cl_2 can be significant only at low pH values (below pH 2); while HOC1 is dominant between pH 3 and 6. Between pH 6 and 9, the relative fraction of HOC1 decreases, while the corresponding fraction of OCl^- increases. The dissociation of HOC1 is also temperature dependent. The effect of temperature is such that at a given pH, the fraction of HOCl will be lower at higher temperatures. Generally, the disinfectant capabilities of HOCl are greater than that of OCl, especially at short contact times (Montgomery, 1985).

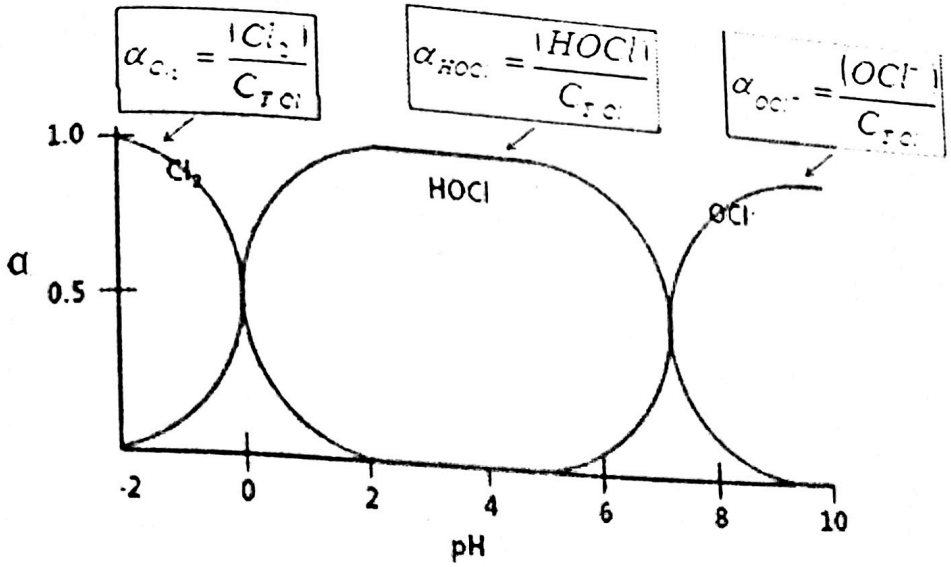


Figure 1: Distribution diagram for chlorine species at 25°C (Total Chlorine, $C_T = 10^{-3}$ M)

Reactions with Impurities in Water:

Reactions with Ammonia: Free chlorine reacts readily with ammonia and certain nitrogenous compounds to form what are collectively known as "combined chlorine". The inorganic chloramines consist of three species: monochloramine (NH_2C1), dichloramine ($NHC1_2$) and trichloramine or nitrogen trichloride ($NC1_3$). The presence and concentrations of these combined forms depend on a number of factors including the ratio of chlorine to ammonia-nitrogen, chlorine dose, temperature, pH and alkalinity.

$$NH_3 + HOC1 = NH_2C1 + H_2O \quad (5)$$
$$NH_2C1 + HOC1 = NHC1_2 + H_2O \quad (6)$$
$$NHC1_2 + HOC1 = NC1_3 + H_2O \quad (7)$$

In addition to chlorinating ammonia, chlorine also reacts to oxidize ammonia to chlorine-free products (e.g., nitrogen gas and nitrate) as shown below.

$$3\,Cl_2 + 2\,NH_3 = N_2(g) + 6\,H^+ + 6\,Cl^- \quad (8)$$
$$4\,Cl_2 + NH_3 + 3\,H_2O = 8\,Cl^- + NO_3^- + 9\,H^+ \quad (9)$$

The mono- and dichloramines have significant disinfecting power and are therefore of interest in the measurement of chlorine residuals. Combined chlorine in water supplies may be formed in the treatment of raw waters containing ammonia; chlorinated wastewater effluents, as well as certain chlorinated industrial effluents normally contain only combined chlorine.

Reactions with Other Impurities: Chlorine combines with various reducing agents and organic compounds thus increasing the chlorine demand which must be satisfied before chlorine is available to accomplish disinfection.

Fe^{2+}, Mn^{2+}, NO_2^-, and H_2S are examples of inorganic reducing agents present in water supplies that will react with chlorine. Chlorine can react with phenols to produce mono-, di-, or trichlorophenols, which can impart tastes and odors to waters. Chlorine also react with humic substances present in water to form trihalomethanes (THMs, e.g., chloroform, bromoform, etc.) which are suspected human carcinogens (Note: According to USEPA, maximum allowable level of THMs in drinking water is 100 µg/L).

Break Point Chlorination:

If chlorine is added to water containing reducing agents and ammonia (either naturally present or added to water to produce combined chlorine), a hump-shaped breakpoint curve is produced.

The different segment of the curve is described below:

a. If the water is free of ammonia and other compounds that may react with chlorine, the application of chlorine will yield free available chlorine residual of same concentration. This is denoted by the *"no demand line"* or the "zero demand line".

b. Chlorine first reacts with reducing agents such as H_2S, Fe^{2+}, Mn^{2+} and develops no measurable residual as shown by the portion of the curve from A to B.

c. Addition of chlorine beyond point B results in forming mainly mono- and di-chloramines. With mole ratios of chlorine to ammonia up to 1:1 [i.e., Cl_2:NH_3-N = 1:1], both mono and di-chloramines are formed. Chloramines thus formed are effective disinfectants.

d. Further increase in the mole ratio of chlorine to ammonia result in formation of some trichloramine and oxidation of part of ammonia to N_2 and NO_3^- (see Eq. 8, 9). These reactions are essentially complete when 1.5 mole of chlorine has been added for each mole of ammonia nitrogen originally present in water [i.e., Cl_2:NH_3-N = 1.5:1]. This is represented by the portion of the curve from C to D.

e. Addition of chlorine beyond point D would produce free chlorine residuals and is referred to as *"breakpoint chlorination"*. In other words, chlorination of water to the extent that all ammonia is converted to N_2 or higher oxidation state is referred to as *"breakpoint chlorination"*.

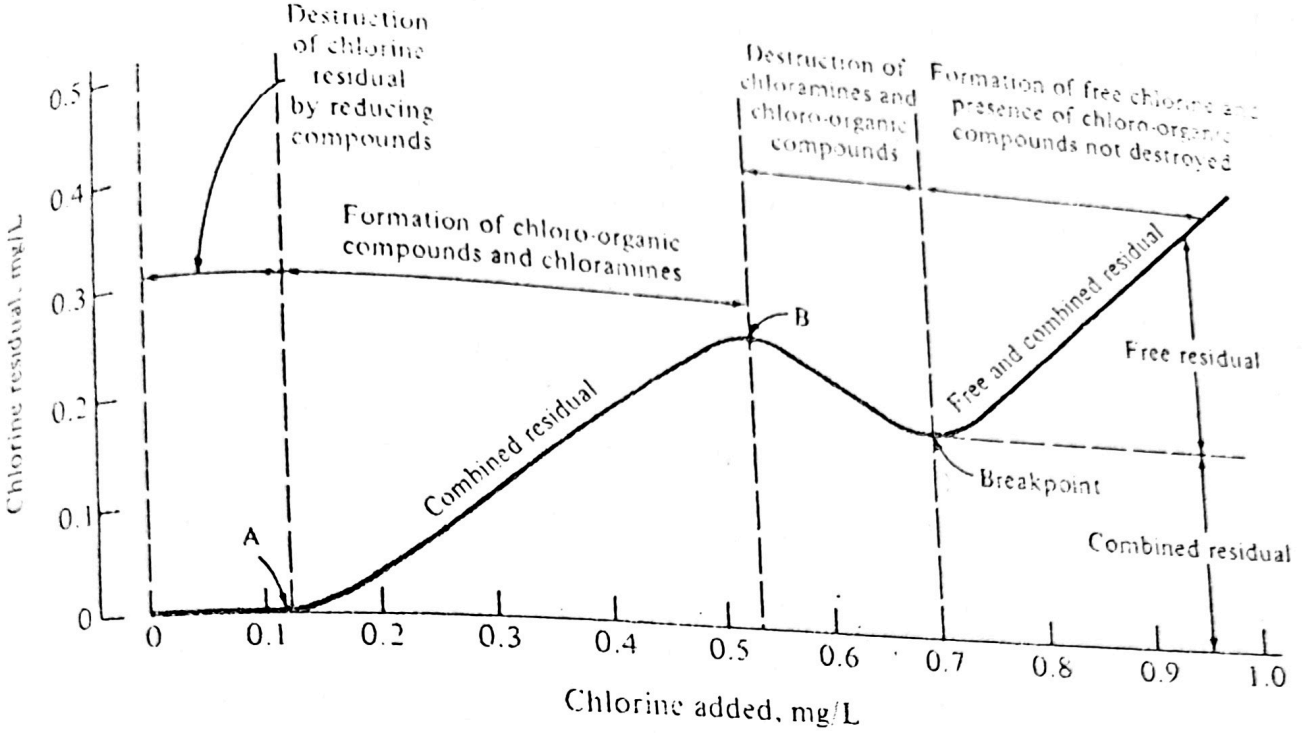


Figure 2: Generalized curve obtained during breakpoint chlorination (Source: Peavy and Rowe, 1985)

Breakpoint chlorination is required to obtain a free chlorine residual for better disinfection if ammonia is present in a water supply. While free chlorine residuals have good disinfecting powers, they are usually dissipated quickly in the distribution system. For this reason, final treatment with ammonia if often practiced to convert free chlorine residuals to longer-lasting combined chlorine residuals. The difference between the amount of chlorine added to the water and the amount of residual chlorine (i.e., free and combined available chlorine remaining) at the end of a specified contact period is termed as *"chlorine demand"*.

REAGENTS:

1. Starch Indicator
2. Standard 0.001N Sodium thiosulfate
3. Potassium Iodine crystal
4. Cone. Hydrochloric Acid
5. Chlorine water

APPARATUS:

1. Erlenmeyer flask (250 ml) : 1 no.
2. Bottle (8-oz) : 6 nos.
3. Beaker (250 ml) : 1 no.
4. Measuring cylinder : 1 no.
5. Dropper : 1 no.
6. Stirrer : 1 no.

PROCEDURE:

1. Place 200-ml portion of the water to be chlorinated in each of six 250-mL flasks.
2. Add required quantity (as instructed by your teacher) of "chlorine water" (stock solution of bleaching powder in water) in each of the flasks. The chlorine content of the "chlorine water" (determined earlier in the laboratory) would be provided to you by your teacher. Calculate the chlorine dose for each of the six flasks.
3. Shake each flask gently and allow to stand for 30 minutes.
4. Determine residual chlorine of water from each flask by the starch-iodine method as described below:

Starch-Iodine Method:

The starch-iodine method is based on the oxidizing power of free and combined chlorine residuals to convert iodide ion into free iodine as shown below.

$$Cl_2 + 2I^- = I_2 + 2\,Cl^-$$

I_2 + starch → blue color (qualitative test for the presence of Chlorine)

In the starch-iodine method, the quantity of chlorine residuals is determined by measuring the quantity of iodine released by titration with a reducing agent sodium thiosulfate ($Na_2S_2O_3$). The end point of titration is indicated by the disappearance of blue color.

$$I_2 + 2\,Na_2S_2O_3 = Na_2S_4O_6 + 2\,NaI$$

Or,
$$I_2 + 2\,S_2O_3^{2-} = S_4O_6^{2-} + 2I^-$$

The procedure to be followed in the laboratory is described below:

(a) Place 200 ml of the sample in an Erlenmeyer flask. Chill to 20^0C or lower by immersing the flask in a bath of ice-water.
(b) Add a small crystal of potassium iodine and 1 mL of concentrated hydrochloric acid to the water.
(c) Add 1 ml of starch solution to the water. A blue color shows the presence of chlorine in the water.
(d) Titrate the solution with standard sodium thiosulfate ($Na_2S_2O_3$) solution until the blue color just disappears.
(e) Record the quantity (in mL) of sodium thiosulfate ($Na_2S_2O_3$) solution used.

CALCULATION:

Residual chlorine (mg/L) = mL of 0.001N Sodium thiosulfate used × Multiplying Factor (MF)

where, MF = normality of $Na_2S_2O_3$ × equivalent wt. of Cl_2 × 1000 / (ml of sample taken)
$= 0.001 \times 35.45 \times 1000/200 = 0.1773$

ASSIGNMENT QUESTIONS

(1) What are the major disadvantages of chlorination? Name some of the alternate disinfectants.

(2) You would like to perform chlorination to a water sample with pH 7.5. At this pH, what would be the relative proportions of HOC1 and OCl^- (see Figure 1). What kind of change in pH would you propose in order to increase the relative proportion of HOC1, which is a better disinfectant?

(3) Schematically draw a "chlorine residual" versus "chlorine dose" curve for a water sample with no ammonia or organic matter.

Course Number : CE 332
Course Name : Environmental Engineering Lab
Course Credit : 1.50

Experiment No. 14
Determination of Arsenic in Water

INTRODUCTION

Arsenic, the 20th most abundant element in the earth's crust and the 12th most abundant element in the biosphere, is a common trace inorganic contaminant in drinking water and is identified as a significant health risk. Arsenic is known for its high toxicity and its ability to induce cancer after long-term ingestion. Presence of elevated levels of arsenic in groundwater (especially from shallow aquifer) has become a major concern in Bangladesh. Arsenic pollution of groundwater is particularly challenging in Bangladesh since tubewell water extracted from shallow aquifers is the major source of drinking water for most of its population. The rural water supply is almost entirely based on groundwater supply through use of hand pump tubewells; the urban supply is also heavily dependent on groundwater.

Awareness about the presence of arsenic in groundwater has been growing since late 1993 when arsenic was first detected in the district of Chapai Nawabganj bordering the West-Bengal district of India. Since then higher levels of arsenic (exceeding the WHO standard of 0.01 mg/L and Bangladesh standard of 0.05 mg/L) have been detected in many regions of the country. Affected areas and estimates of affected population are being updated, as more data are becoming available. Out of 64 administrative districts of Bangladesh, arsenic contamination has so far been reported in 61 districts and an estimated 20 million people are at risk of arsenic toxicity. This has lowered the population coverage by safe water supply to an estimated 80 percent from an impressive figure of nearly 98 percent. The southern and northeastern districts are the worst affected by arsenic pollution. Figure 1 shows a distribution of arsenic concentration in groundwater of Bangladesh.

In Bangladesh, the arsenic in groundwater is of geologic origin and is probably only apparent now because it is only the last 20-30 years that groundwater has been extensively used for drinking in rural areas. Weathering of arsenic-rich base metal sulfides in the upstream of the Ganges basin appears to be a major source of arsenic-rich iron oxyhydroxides in the sediments of Bangladesh. Arsenic-rich iron oxyhydroxides appear to be the major source of arsenic, from which arsenic is released as a result of dissolution and desorption. Reducing environment in the alluvial aquifer, primarily due to the presence of organic matter, promotes dissolution of iron oxyhydroxides and release of arsenic. Use of phosphate fertilizer can potentially enhance release of arsenic as a result of replacement of arsenic by phosphate ions on the adsorption sites of iron oxyhydroxides. Natural and anthropogenic processes that may lead to release/ mobilization of arsenic in the subsurface are being investigated.

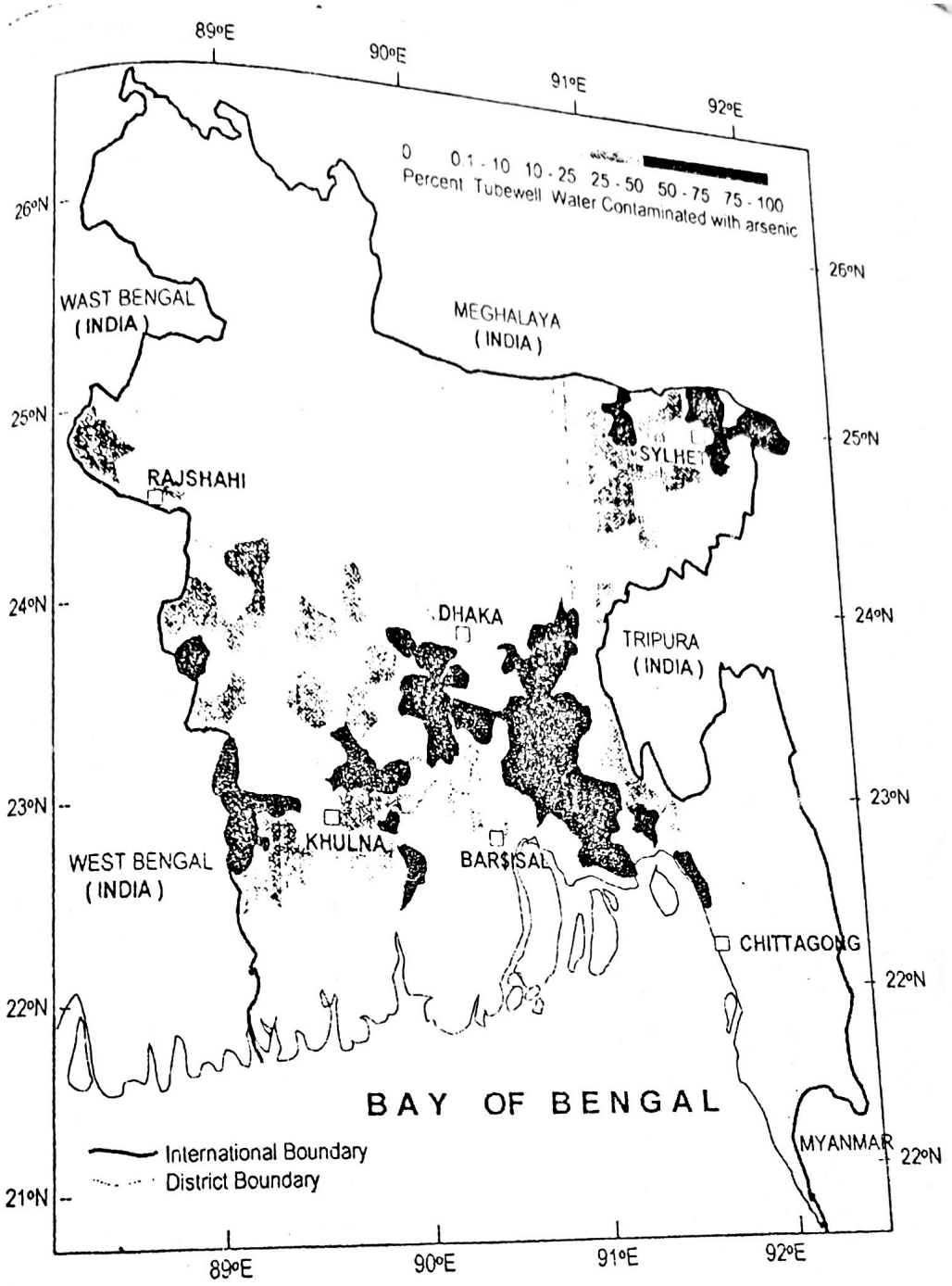


Figure 1. Distribution of Arsenic in Groundwater of Bangladesh

According to ECR 1997, drinking water standard for arsenic in Bangladesh is 50 µg/L (or 0.05 mg/L). The WHO guideline value for arsenic in drinking water is 10 µg/L and the USEPA is also planning to revise its standard from 50 µg/L to 10 µg/L.

ARSENIC CHEMISTRY

Arsenic occurs in water in several different forms, depending upon the pH and the redox potential, E_h. Some of the most important compound and species are shown in Table 1. In groundwater, arsenic primarily exists as inorganic arsenic. Inorganic trivalent arsenic, [As(III)] or arsenite is the dominant form in reducing environment; while inorganic pentavalent arsenic

[As(V)] or arsenate is the dominant form in oxidizing or aerobic environment. In groundwater environment where the conditions are mostly reducing, a significant part of the arsenic exists as As(III). As shown in Table 1, in the pH range of most groundwater (i.e., pH 6 - 9), dominant chemical form of As (III) is H_3AsO_3; while dominant chemical forms of As(V) in this pH range are: H_2AsO_4, $HAsO_4^{2-}$.

Table 1: Arsenic compounds and species and their environmental and toxicological importance in water

Compounds	Examples	Environmental Significance/ Dominant pH region	Toxicity
Arsine	As^3	Minor Importance	Most toxic As species
Element Arsenic	As	Minor Importance	Least toxic As species
Trivalent Arsenic	As(III)	Anaerobic	10 times more toxic than As (V)
Arsenite, Inorganic	H_3AsO_3, $H_2AsO_3^-$, $HAsO_3^{2-}$, AsO_3^{3-}	pH = 0-9 pH = 10-12 pH = 13 pH = 14	
Methylated As (III) Organo-As(III)		Minor Importance	Less toxic than Inorganic As(III)
Pentavalent arsenic	As(V)	Aerobic	10 times less toxic than As(III)
Arsenate, Inorganic	H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, AsO_4^{3-}	pH = 0-2 pH = 3-6 pH = 7-11 pH = 12-14	
Methylated As (V) Oragano-As(V)		Minor Importance	Less than Inorganic As(V)

HEALTH EFFECTS OF ARSENIC

In a population drinking arsenic contaminated water, a great variety of specific as well as non-specific symptoms may be observed. Table 2 lists some of the effects of arsenic reported to be due to exposure through drinking water.

In a study by the National Institute of Preventive and Social Medicine (NIPSOM), arsenic related diseases (arsenicosis) have been identified in 37 districts (Ahmad et al., 1998). A total of 6000 cases were identified in 162 villages in the 37 districts, mostly in the rural areas. Three stages of manifestations of chronic arsenicosis were observed in the study (Ahmad et al., 1998), but most of the patients were found in the first and second stages. The most common presentations were melanosis, keratosis, hyperkeratosis and depigmentation. Cancers were found among 0.8% and actinic keratosis and Bowen's diseases were observed among 3.1% of the cases. It is important to note that the study found that the cases at initial and second stages of toxicity showed improvement when patients stopped taking arsenic contaminated water and increased intake of protein rich food, vitamin A, E and C.

Table 2: Toxicological effects due to exposure to high arsenic concentration in drinking water (WHO, 1996; Khan, 1997)

Effect	Symptoms	Remarks
Blackfoot Disease Arsenical Dermatosis	Dermal lesion, Peripheral neuropathy Keratosis, Hyperkeratosis, Hyperpigmentaion	May necessitate operation
None specific	Nausea, Abdominal Pain, Diarrhoea, Vomiting, Conjunctivitis, Oedema	Mainly due to acute intoxication
Pregnancy disorders	Spontaneous abortions, miscarriages	-
Heart disease	Coarctation (narrowing) of aorta, cardiovascular disturbance	Among children
Cancer	Bladder, Kidney, Skin & Lungs, Liver & Colon	-
Mortality	-	Mainly due to cancer

ANALYTICAL METHODS FOR MEASURING ARSENIC

Analytical methods for measuring arsenic may be categorized as follows:

(1) Inductively coupled plasma (ICP) method
(2) Graphite furnace atomic absorption spectrophotometry
(3) Hydride generation atomic absorption spectrophotometry
(4) Hydride generation-scraper-spectrophotometry (SDDC method)
(5) Hydride generation-scraper-indicator paper-field kit

The first three methods involve high-cost equipment and provide more accuracy and lower detection limit (in the range of 1 µg/L). The last two methods are relatively low cost methods. The principle employed in these two methods is explained Fig. 2.

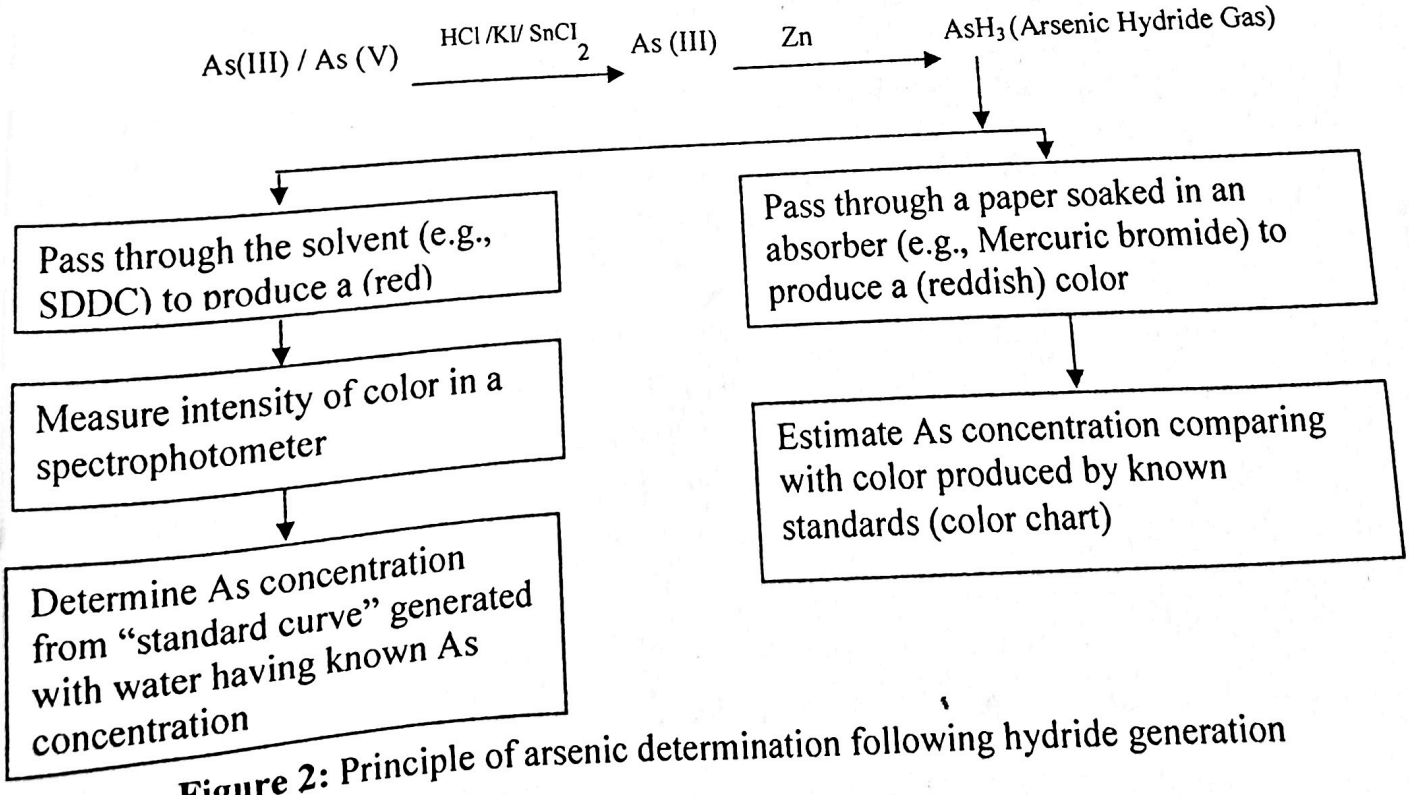


Figure 2: Principle of arsenic determination following hydride generation

quations 1-3 show reactions for arsine generation and formation of colored complex.

$$+ \quad 2H^+ = Zn^2 + 2H \quad (1)$$

$$\text{metal)} + 6H = AsH_3 + 3H_2O \quad (2)$$

$$AsO + \text{SDDC/Bromide} = \text{Colored Complex} \quad (3)$$

AsH_3

RSENIC DETERMINATION

HYDRIDE GENERATION-SCRAPER-SPECTROPHOTOMETRY, *and* HYDRIDE GENERATION-SCRAPER-INDICATOR PAPER METHOD

***reparation of Sample*:** Arsenic is often present in very small concentration in water. herefore, water samples are often *pre-concentrated* in order to increase the quantity of senic in a given volume. Pre-concentration is usually done by acidifying 200 to 500 ml ater sample and then reducing the volume to about 35 ml in a water bath.

***recaution*:** Arsenic is often found in groundwater with high concentration of iron (Fe). After xtraction, Iron present in groundwater in ferrous (Fe^{2+}) form is converted to ferric (Fe^{3+}) form contact with atmospheric oxygen and forms ferric hydroxide $Fe(OH)_3$ (s) flocs. Arsenic, pecially As(V), is strongly adsorbed onto these ferric hydroxide flocs. Therefore, care should taken while taking water sample from a bottle in which red colored ferric hydroxide flocs are sible. It is better to acidify the bottle first (thus dissolving the ferric hydroxide flocs) and then take ater sample for pre-concentration.

) ARSENIC DETERMINATION: *HYDRIDE GENERATION-SCRAPER-SPECTROPHOTOMETRY:* SILVER DIETHYLDTHIOCARBAMATE (SDDC) METHOD

e method followed in the laboratory is commonly referred to a "*Silver elhyldithiocarbamate Method*" (Standard Method, 307B, 1985). Minimum detectable quantity As for this method is 1 µg. This method essentially involves: (i) conversion (reduction) of arsenic in water into As(III), (ii) generation of arsine gas in the form of arsenic hydride sH_3), (iii) passing of arsine gas through a solution of silver diethyl - dithiocarbamate (SDDC) d producing a red-colored complex; (iv) measurement of absorbance of the colored mplex in a spectrophotometer at 535 nm wavelength; (v) determination of arsenic ncentration from a standard curve (As concentration versus Absorbance) developed with own concentration of arsenic following the same procedure.

EAGENTS

) Hydrochloric acid, (2) Potassium Iodide solution,
) Stannous chloride solution, (4) Metallic zinc,
) Silver diethyl-dithiocarbamate solution in pyridine.

PPARATUS

) Assembly for SDDC method
) Spectrophotometer

CEDURE

Take about 35 ml sample in a clean Arsine generator bottle. Add (with constant stirring) 5 ml concentrated hydrochloride acid (HCl), 2 ml potassium iodide (KI) solution, and 0.40 ml stannous chloride ($SnCl_2$) solution. Wait 15 minutes for reduction of arsenic to the trivalent state [As(III)].

) Soak glass wool in lead acetate (PbAc) solution and put in the scrubber. This is done in order to eliminate interference from sulphide gas by absorbing it in the scrubber. Pipette 4- ml silver diethyl-dithiocarbamate reagent in the absorber tube.

) Add 3 g metallic zinc (Zn) in the generator and connect scrubber-absorber assembly immediately. Make certain that all connections are fitted tightly.

) Allow 30 minutes for complete generation of arsine (according to equations 1, 2). Warm the generator slightly to ensure that all arsine is released. Pour solution from absorber in tubes for measurement of absorbance.

5) Measure absorbance of the solution at 535 run in a spectrophotometer, using reagent blank as the reference.

6) *Preparation of Standard Curve*: Treat portions of standard solution containing 0. 1.0, 2.0, 5.0, and 10.0 µg As in the same manner described above. Plot absorbance versus concentration of As in the standard and develop a standard curve.

7) Concentration of Arsenic:

$$\mu g\ As/L = (\mu g\ As \text{ from standard curve}) \times 1000 / (\text{volume of sample})$$

(b) ARSENIC DETERMINATION USING *HYDRIDE GENERATION SCRAPER-INDICATOR PAPER METHOD:* MODIFIED MERCURIC BROMIDE STAIN METHOD *(JALIL , 1998)*

A simple and reasonably accurate method for arsenic measurement has been developed in the Environmental Engineering Laboratory, Department of Civil Engineering. BUET (Jalil. 1998). The measurement using the "BUET Arsenic Kit" follows the principle of Mercuric Bromide Stain Method. It involves: (i) reduction of As(V) in water to As(III); (ii) generation of arsenic hydride (AsH_3); (iii) passing arsine gas through a filter paper soaked in mercuric bromide and producing a yellowish color on the paper, and (iv) determination of arsenic concentration by comparing the produced color with a standard colored chart.

REAGENTS

(1) Hydrochloric acid; (2) Potassium Iodide solution;
(3) Stannous chloride solution; (4) Zinc powder
(5) Filter paper soaked in mercuric bromide solution

APPARATUS

Assembly for "BUET Arsenic Kit"

PROCEDURE

(1) Take 50-ml water sample in the clean arsine generator bottle. Add 5 ml HCl, 1 ml KI and 0.2 ml $SnCl_2$ solution. After each addition, shake the generator for mixing the contents. Wait 15 minutes for reduction of As(V) to As(III).
(2) Take a mercuric bromide soaked filter paper and air-dry it.

Insert the dried paper in its proper position in the rubber-cork assembly.
Add 1 g powder zinc into the generator and immediately close the generator bottle tightly with the rubber-cork assembly.
Allow 30 minutes for complete reaction.
Take the filter paper out from the rubber-cork assembly.
Compare the color of the filter paper with a standard "color chart". The color chart is prepared by treating 5, 10, 20, 30, 50, and 70 µg/L standard arsenic solutions in the same manner described above. If the color produced by the sample does not match any color in the chart, arsenic concentration of the sample is estimated by interpolation between colors in the chart.

SIGNMENT QUESTIONS

In which chemical form arsenic is likely to exist in groundwater? Explain.
In determination of arsenic (following the methods described here), why the water samples are acidified and often pre-concentrated before actual determination of arsenic?
What is the basic difference between the two arsenic determination methods - SDDC method and Mercuric Bromide Stain method, described above?

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se Number : CE 332
se Name : Environmental Engineering Lab
se Credit : 1.50

Experiment No. 15

termination of pathogenic microorganisms (Total Coliform and Fecal Coliform) in water and wastewater

ODUCTION

iety of different microorganisms are found in untreated water. Most of these organisms do se a health hazard to humans. Certain organisms, referred to as pathogens, cause disease to ns which include species of bacteria, viruses and protozoa. These organisms are not native uatic systems and usually require an animal host for growth and reproduction. They can, vér, be transported by natural water systems, thus becoming a temporary member of the ic community. Many species of pathogens are able to survive in water and maintain their tious capabilities for significant periods of time. From the perspective of human use and mption, the most important organisms in water are the pathogens. Table 1 lists the more non waterborne diseases and the organisms that cause the diseases as well as the disease ptoms.

e 1: Waterborne Diseases

terborne iseases	Causative Organism	Source of organism in water	Symptom
roenteritis	*Salmonella* (bacteria)	Animal or human feces	Acute diarrhea and vomiting
yphoid	*Salmonella typhosa* (bacteria)	Human feces	Inflamed intestine, enlarged spleen, high temperature – fatal
ysentery	*Shigeila* (bacteria)	Human feces	Diarrhea- rarely fatal
holera	*Vibrio Comma* (bacteria)	Human feces	Vomiting, severe diarrhea, rapid dehydration, mineral loss - high mortality
fectious epatitis aundice)	Virus	Human feces	Yellowed skin, enlarged liver, abdominal pain - low mortality, lasts up to four months
moebic ysentery	*Entamoeba histolytica* (protozoa)	Human feces	Mild diarrhea, chronic dysentery
iardiasis	Giardia lamblia (protozoa)	Suspect wild animal feces	Diarrhea, cramps nausea and general weakness - lasts 1week to 30 weeks, not fatal

ARACTERSTICS OF PRIMARY WATERBORNE PATHOGENS

major characteristics of bacteria, viruses and protozoa are briefly described below:

teria:

y are unicellular prokaryotic organisms and arc the lowest form of life capable of

synthesizing protoplasm from the surrounding environment. They can grow on artificial laboratory media. Cell multiplication is usually by binary fission.

Viruses:
They are the smallest biological structures known to contain all the generic information necessary for their own reproduction. They require a host in which to live. They do not grow on artificial media, rather require living cells within which they are reproduced. Electron microscope is required see viruses. Multiplication takes place by replication.

Protozoa:
They are unicellular eukaryotic organisms and are more complex in their functional activity than bacteria and viruses. Some can be grown on artificial media and some are parasites. Reproduction is by asexual and sexual processes.

INDICATOR ORGANISMS

One might assume that a water sample is analyzed to search for pathogenic organisms. This is not true, however for the following reasons:

- Pathogens are likely to gain entrance sporadically, and they do not survive for very long period of time; consequently they could be missed in a sample submitted to the laboratory.
- If they are present in very small numbers, pathogens are likely to escape detection by laboratory procedures.
- Although it is possible to detect the presence of various pathogens in water, the isolation and identification of many of these is often extremely complicated and seldom quantitative.
- Analysis of water for all known pathogens would be a very time-consuming and expensive proposition.

Tests for specific pathogens are usually made only when there is a reason to suspect that those particular organisms are present. At other times, the microbiological quality of water is checked using indicator organisms. An indicator organism is one whose presence presumes that contamination has occurred and suggests the nature and extent of the contaminations.

CHOICE OF INDICATOR ORGANISMS

It is known that most pathogens that are likely to be transmitted via the water route are shed in human and/or animal feces. Hence, and indicator organism should be a microorganism whose presence is the evidence of fecal contamination of warm blooded animals. Indicators may be accompanied by pathogens, but typically do not cause disease themselves. The ideal indicator organism should have the following characteristics:

- be applicable to all waters
- always be present when pathogens are present
- always be absent where pathogens are absent
- numbers should correlate the degree of pollution
- be present in greater number than pathogens

- there should be no after-growth or re-growth in water
- there should be greater or equal survival time than pathogens
- be easily and quickly detected by simple laboratory tests
- should have constant biochemical and identifying characteristics
- harmless to humans

organisms or group of organisms meet all of these criteria; but the coliform bacteria fulfill st of them, and this group is most common indicator in use today. Each person discharges ut 100 to 400 billion coliforms each day. Thus, the presence of coliform organisms is taken an indication that pathogenic organisms may also be present, and the absence of coliform anisms is taken as an indication that the water is free from pathogens. Total coliforms are ined as gram negative bacteria which ferment lactose at 35°C or 37°C, with the production of d, gas and aldehyde within 24 or 48 hours. They are cytochrome oxidase negative and non-re forming.

Total coliform (TC) = Fecal coliform (FC) + Non-fecal coliform.

cal coliforms (thermo-tolerant coliform) are a subgroup of total coliforms, which live in the rm blooded animals and have the same properties as the total coliforms but tolerate and grow high temperature.

ETERMINATION METHODS FOR INDICATOR ORGANISMS

) *Multiple Tube (MT) Method:*

he multiple tube method is applicable to all kinds of water samples. It can cope with clean, lored, or turbid waters containing sewage or sewage sludge, or mud and soil panicles, ovided that the bacteria are homogenously distributed in the prepared test samples. neoretically, the technique is sufficiently sensitive to measure low levels of bacteria in water mples, although bigger containers to take larger volumes of sample are needed to be used as lture vessels. However for normal water analysis, 10 ml is usually the largest volume used. his method is often preferred by microbiologists.

) *Membrane Filter (MF) Method:*

contrast to the multiple-tube (MT) method, the membrane filter (MF) method gives a direct unt of total coliforms and fecal coliforms present in a given sample of water. The method is sed on the filtration of a known volume of water through a membrane filter consisting of a llulose compound with a uniform pore diameter of 0.45 μm; the bacteria are retained on the rface of the membrane filter. When the membrane containing the bacteria is incubated in a erile container at an appropriate temperature with a selective differential culture medium, haracteristic colonies of coliforms and fecal coliform develop, which can be counted directly. his technique is popular with environmental engineers.

his method is not suitable for turbid waters, but otherwise it has several advantages. Its articular advantages and limitations are as follows:

dvantages:

- Results are obtained more quickly as the number of coliform can be assessed in less than 24 hours, whereas the multiple tube technique requires 48 hours both for a negative or a presumptive positive test.
- Saving in work, certain supplies and glassware.

- This method gives direct results.
- Easy to use in laboratories, or even in the field if portable equipment is used.

dvantages:

- High turbidity caused by clay, algae, etc. prevents the filtration of a sufficient volume of water for analysis and it may also produce a deposit on the membrane which could interfere with bacterial growth.
- Presence of a relatively high non-coliform count may interfere with the determination of coliforms.
- Waters containing particular toxic substances which may be absorbed by the membranes can affect the growth of the coliforms.

STS FOR DETERMINATION OF INDICATOR ORGANISMS

) Multiple Tube Test

ılture Media:

ɔmmercially available dehydrated media simplify the preparation of culture broths, and are erefore recommended for laboratory work. Various manufactures produce these media as ɔwders, which can then be easily weighted out, dissolved in distilled water, and dispensed into ılture tubes prior to sterilization.

several different culture media are available for the presumptive test, for example: (i) lauryl yptose broth (LTB), (ii) MacConkey broth, and (iii) lactose broth. These three media are ɔmmon in use in many countries. The selectively of MacConkey broth and LTB depends espectively on the presence of bile salts and the surface-active agents, lauryl sulfate; lactose roth is a non-selective medium.

ıs a confirmatory medium for total coliforms (TC), brilliant green lactose bile broth (BGB) is nost widely used. To confirm the presence of fecal coliform (FC), either BGB broth or Escherichia coli (EC) broth is used.

Preparation of Media:

Media should be prepared in accordance with the manufacturer's instructions, as follows:

- Dissolve the stated amount of the dehydrated medium in distilled water to obtain the double-strength or single-strength presumptive medium (for confirmatory analysis, only singe-strength medium is used).
- Dispense the requisite volume into culture tubes containing an inverted Durham tube and cap the culture tubes.
- Sterilize in an autoclave or pressure cooker at 114°C for 10 minutes (or in accordance with the manufacture's specifications). It is particularly important that media containing disaccharides, e.g. lactose, are not autoclaved at higher temperatures.
- The sterilized medium should be stored at room temperature to maintain sterility. Since several dyes are light-sensitive, the solution should be protected from exposure to light.

Test Procedure:

Figure 1 shows the procedure involved in Multiple Tube (MT) Test of water and wastewater samples, together with the appropriate incubation times and temperatures. The procedure to be

used for testing relatively unpolluted water, such as treated water from waterworks, is described below.

(A) Remove the paper wrapping from the sample bottle.

(B) With the stopper in position, shake the bottle vigorously to achieve a homogeneous disoperation of bacteria. If the bottle is completely full of water, remove the stopper and discard about 20-30 ml of water, then replace the stopper and shake. This ensures thorough mixing.

(C) With a sterile 10 ml pipette, modulate 10 ml of the sample into each of five tubes containing 10 ml of presumptive broth (double strength). It is advisable to shake the tubes gently to distribute the sample uniformly throughout the medium.

(D) Incubated the tubes at 35°C or 37°C for 24 hours.

(E) At the end of the 24-hour incubation period, observe each tube for the presence of gas. Gas, if present, can be seen in the Durham tube; if none is visible, ge3ntly shake the tube. If any effervescence (foam, streams of tiny bubbles) is observed, the tube should be considered positive.

(F) Enter the number of positive tubes after 24 hours in table.

(G) Re-incubate negative tubes for a further 24-hour period. At the end of this period, check the tubes again for gas production as in (E) above. Gas production at the end of either 24 or 48 hours incubation is pressured to be due to the presence of coliform in the sample.

(H) Enter the number of positive tubes after 48 hours in the table.

(I) The confirmatory test should be carried out at the end of both the 24-hour and the 48-hour incubation. Using a loop, transfer one or two drops from each presumptive positive tube to a corresponding sterile confirmative 10-ml tube containing, e.g. BGB broth. Before each transfer, sterilize the inoculation loop by flaming and allow it to cool.

(J) If the presence of fecal coliforms is also to be investigated, subcultures in two tubes containing confirmative broth (e.g. BGB broth) should be prepared from each presumptive positive tube. EC medium is preferred in some areas for confirmation of fecal coliforms.

K) To confirm the presence of coliforms, incubate one subculture tube from each presumptive positive tube for 48 hours at 35°C or 37°C.

L) Check the tubes at the end of the 48 hour incubation period; the presence of gas coliforms that coliforms are present in the sample. Enter the results in the table.

M) To confirm the presence of fecal coliforms, incubate a second subculture tube from each presumptive positive tube for 24 hours at 44±0.5°C.

N) If, at the end of 24 hour incubation, gas is present in the tubes, the presence of fecal coliforms is confirmed.

Determination of MPN :

For treated water, where five 10 ml portions are portions are inoculated, the MPN can be found from the test results by means of Table 2. An example is given in Fig 2. To show how the test results are interpreted. It can be seen that three confirmed positive tubes have been obtained for the total coliform group. From Table 1, the corresponding value of the MPN can be seen to be 9.2 total coliforms per 100 ml sample. As regards the test for fecal coliforms, there was only one confirmed positive tube. Consequently, the value of the MPN for this group is 2.2 per 100 ml.

Table 2: MPN of various combinations of +ve and –ve results when five 10-ml portions are used

Number of the tubes giving positive reaction out of five 10-ml tubes	MPN
0	0
1	2.2
2	5.1
3	9.2
4	16.0
5	Intermediate

B) Membrane Filter Method:

Volume of water Sample for Filtration:

Since the filtration area is relatively small, it can only support the growth of a limited number of colonies. The optimum number is between 20 and 80 colonies with a maximum of 200. If this figure is exceeded, very small colonies or super imposed colonies may develop, or growth inhibition due to overpopulations may result. The choice of the volume of sample to be filtered will depend on the type of water. As a general rule, the following filtration volumes (Table 3) should be employed.

Table 3: Volume of water to be filtered for various types of water

Type of Water	Volume of sample to be filtered (ml)
Good quality treated water	50-100
Untreated Drinking Water	10-50
Surface Water	1-10

If the origin of the sample is unknown and its probable bacterial content is uncertain, water volumes differing by a factor of ten should be filtered in order to find the appropriate range for the analysis. If the volume to be filtered is less than 10 ml, at least 20 ml of sterile dilution water should be placed in the funnel prior to the filtration.