13.0 EXPERIMENT ON DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND

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13.0 EXPERIMENT ON DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND

PREAMBLE:

"**How to determine biochemical oxygen demand in Water and Wastewater**".

Test procedure is in accordance to IS: 3025 (Part 44) - Reaffirmed 2003.

In addition to our Indian Standard, we also discuss in brief regarding the procedure stated in

(1) APHA Standard Methods for the Examination of Water and Wastewater - 20th Edition. Method 5210 B.

(2) Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, USEPA, Method 405.1.

13.1 AIM

To determine biochemical oxygen demand in the given water sample with the stipulations as per **IS: 3025 (Part 44) - Reaffirmed 2003.**

13.2 INTRODUCTION

The biochemical oxygen demand determination is a chemical procedure for determining the amount of dissolved oxygen needed by aerobic organisms in a water body to break the organic materials present in the given water sample at certain temperature over a specific period of time.

BOD of water or polluted water is the amount of oxygen required for the biological decomposition of dissolved organic matter to occur under standard condition at a standardized time and temperature. Usually, the time is taken as 5 days and the temperature is 20°C.

The test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous ion. It also may measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand).

13.2.1 ENVIRONMENTAL SIGNIFICANCE

BOD is the principle test to give an idea of the biodegradability of any sample and strength of the waste. Hence the amount of pollution can be easily measured by it.

Efficiency of any treatment plant can be judged by considering influent BOD and the effluent BOD and so also the organic loading on the unit.

Application of the test to organic waste discharges allows calculation of the effect of the discharges on the oxygen resources of the receiving water. Data from BOD tests are used for the development of engineering criteria for the design of wastewater treatment plants.

Ordinary domestic sewage may have a BOD of 200 mg/L. Any effluent to be discharged into natural bodies of water should have BOD less than 30 mg/L.

This is important parameter to assess the pollution of surface waters and ground waters where contamination occurred due to disposal of domestic and industrial effluents.

Drinking water usually has a BOD of less than 1 mg/L. But, when BOD value reaches 5 mg/L, the water is doubtful in purity.

The determination of BOD is used in studies to measure the self-purification capacity of streams and serves regulatory authorities as a means of checking on the quality of effluents discharged to stream waters.

The determination of the BOD of wastes is useful in the design of treatment facilities.

It is the only parameter, to give an idea of the biodegradability of any sample and self purification capacity of rivers and streams.

The BOD test is among the most important method in sanitary analysis to determine the polluting power, or strength of sewage, industrial wastes or polluted water.

It serves as a measure of the amount of clean diluting water required for the successful disposal of sewage by dilution.

13.3 PRINCIPLE

The sample is filled in an airtight bottle and incubated at specific temperature for 5 days. The dissolved oxygen (DO) content of the sample is determined before and after five days of incubation at 20°C and the BOD is calculated from the difference between initial and final DO.

The initial DO is determined shortly after the dilution is made; all oxygen uptake occurring after this measurement is included in the BOD measurement.

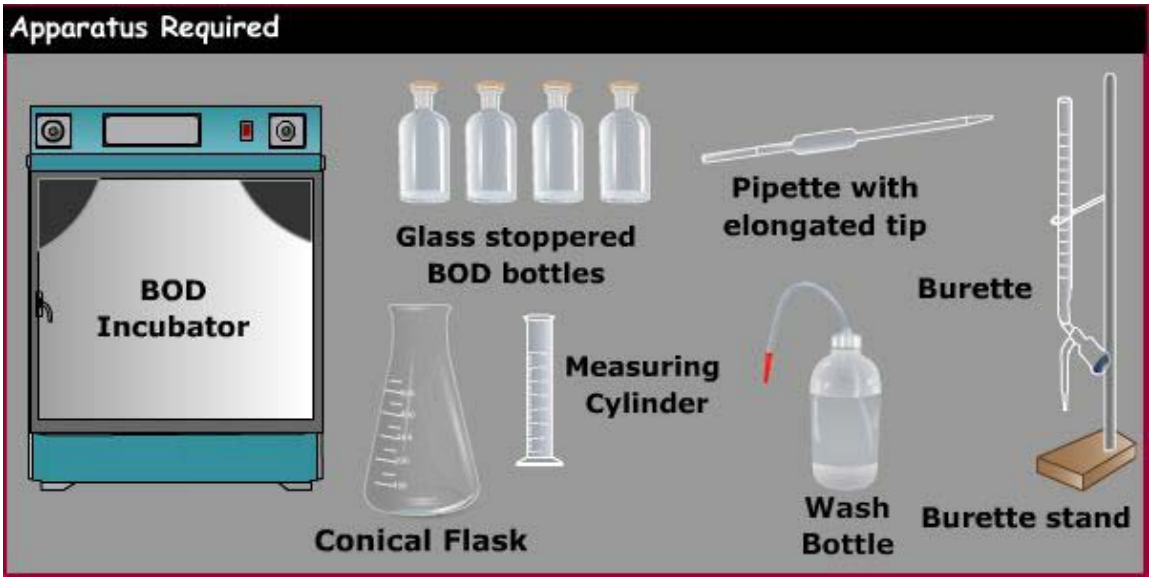
13.4 MATERIALS REQUIRED

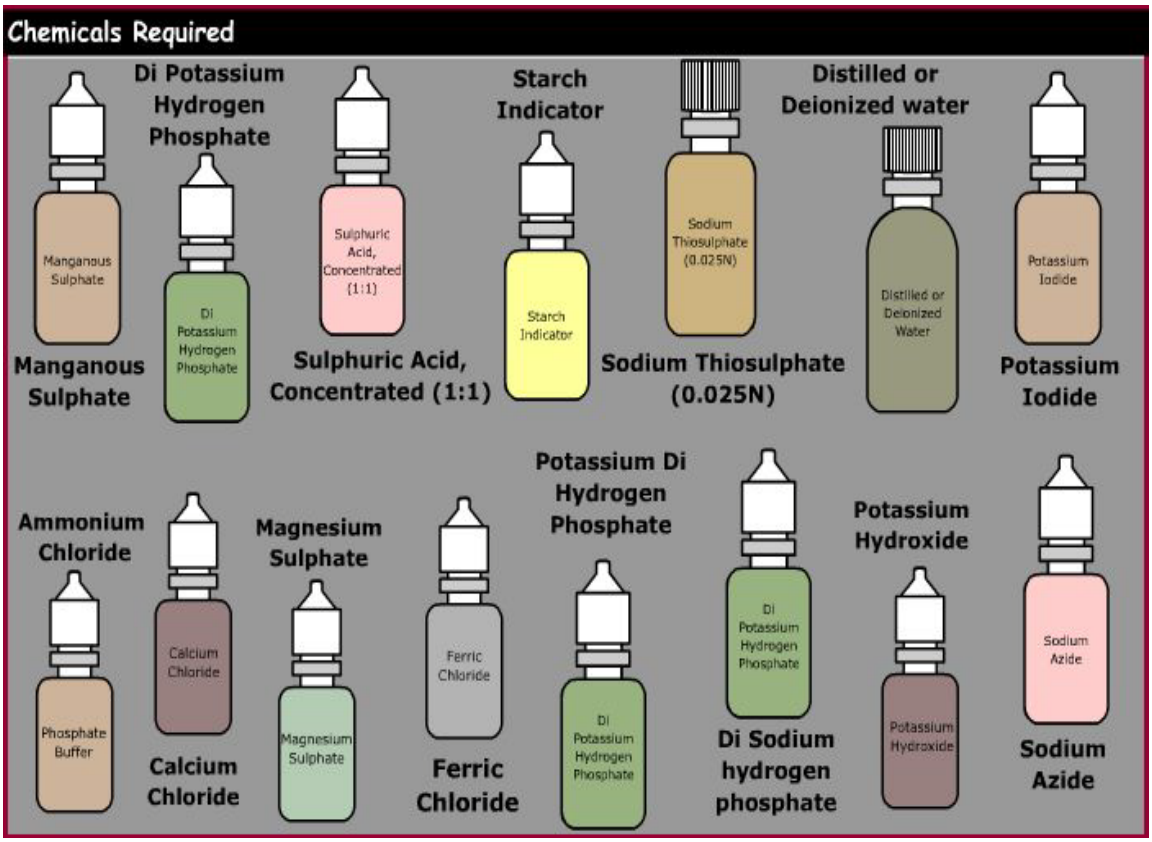
13.4.1 APPARATUS REQUIRED

1. BOD Incubator
2. Burette & Burette stand
3. 300 mL glass stopper BOD bottles
4. 500 mL conical flask
5. Pipettes with elongated tips
6. Pipette bulb
7. 250 mL graduated cylinders
8. Wash bottle

13.4.2 CHEMICALS REQUIRED

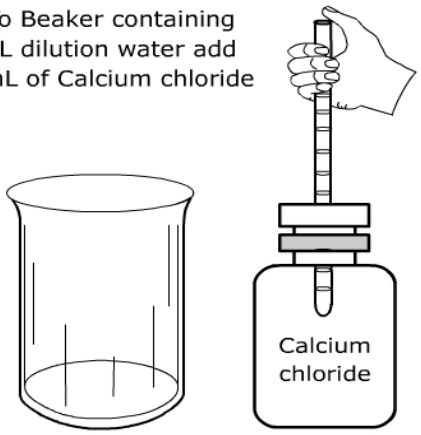
1. Calcium Chloride
2. Magnesium Sulphate
3. Ferric Chloride
4. Di Potassium Hydrogen Phosphate
5. Potassium Di Hydrogen Phosphate
6. Di sodium hydrogen phosphate
7. Ammonium Chloride
8. Manganous sulphate
9. Potassium hydroxide
10. Potassium iodide
11. Sodium azide
12. Concentrated sulfuric acid
13. Starch indicator
14. Sodium thiosulphate
15. Distilled or deionized


Apparatus Required
BOD Incubator
Glass stoppered BOD bottles
Pipette with elongated tip
Burette
Measuring Cylinder
Conical Flask
Wash Bottle
Burette stand


Chemicals Required
Manganous Sulphate
Di Potassium Hydrogen Phosphate
Sulphuric Acid, Concentrated (1:1)
Starch Indicator
Sodium Thiosulphate (0.025N)
Distilled or Deionized water
Potassium Iodide
Ammonium Chloride
Phosphate Buffer
Calcium Chloride
Magnesium Sulphate
Ferric Chloride
Potassium Di Hydrogen Phosphate
Di Sodium hydrogen phosphate
Potassium Hydroxide
Sodium Azide

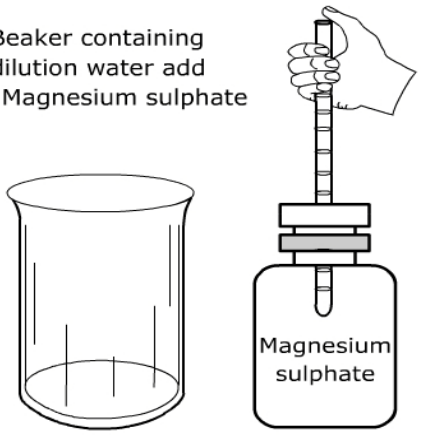
PROCEDURE CHART

To Beaker containing 5L dilution water add 5mL of Calcium chloride



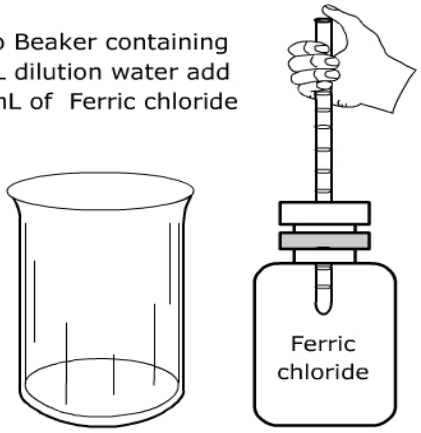
1

To Beaker containing 5L dilution water add 5mL of Magnesium sulphate



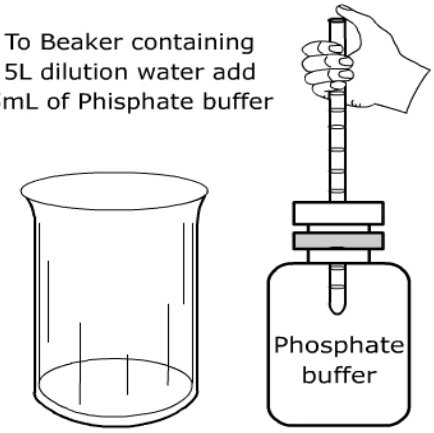
2

To Beaker containing 5L dilution water add 5mL of Ferric chloride



3

To Beaker containing 5L dilution water add 5mL of Phisphate buffer



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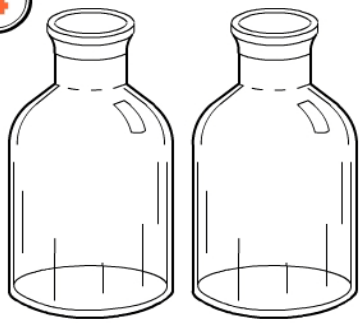
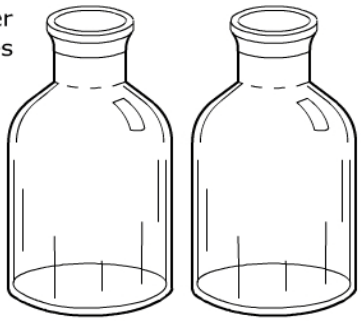


Add 30 mL of field sample and remaining 270mL of dilution water (10 times dilution) to 2 BOD bottles

5

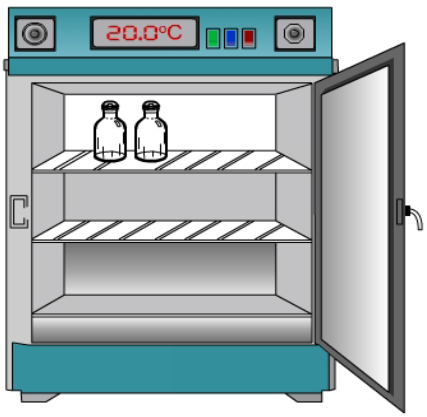
Take 300mL of dilution water in 2 BOD bottles (blank)



6

Each one of the blank and the sample needs to be titrated immediately as per the procedure given in DO chart.

7



Remaining 2 BOD bottles of the blank and the sample needs to be kept in BOD incubator at 20°C for 5 days. After which the blank and the sample needs to be titrated as per the procedure given in DO chart.

13.5 SAMPLE HANDLING AND PRESERVATION

Preservation of sample is not practical. Because biological activity will continue after a sample has been taken, changes may occur during handling and storage.

If Analysis is to be carried out within two hours of collection, cool storage is not necessary. If analysis can not be started with in the two hours of sample collection to reduce the change in sample, keep all samples at 4° C.

Do not allow samples to freeze. Do not open sample bottle before analysis.

Begin analysis within six hours of sample collection

13.5.1 PRECAUTIONS

The following precautions should be observed while performing the experiment:

- Prepare dilution water 3 to 5 days before initiating BOD test to ensure that the BOD of the dilution water is less than 0.2 mg/L. **Discard dilution water if there is any sign of biological growth.**
- The sample should be adjusted to a pH between 6.5 and 7.5, using sulfuric acid for samples with pH in the alkaline side i.e., greater than 7.5 or sodium hydroxide for samples with pH in the acidic side i.e., less than 6.5.
- Add sodium sulfite (Na_2SO_3) to remove residual chlorine, if necessary. Samples containing toxic metals, arsenic, or cyanide often require special study and pretreatment.
- While still letting sample water flow down the tube, slowly pull the tube from the bottom of the bottle and fill the bottle to its brim. Check for bubbles. Carefully stopper the BOD bottle as described above.

13.6 PROCEDURE

For testing the given sample, first the reagents are required to be prepared.

13.6.1 PREPARATION OF REAGENT

a) Manganous Sulphate Solution

Dissolve Manganese Sulphate

→ 480g of $MnSO_4.4H_2O$ (or)

→ 400g of $MnSO_4.2H_2O$ (or)

→ 364 g of $MnSO_4.H_2O$

in freshly boiled and cooled distilled water, filter the solution and make up to 1000 mL (One litre). In this experiment, we are using Manganese sulphate Mono hydrate.

Take 364g $MnSO_4.H_2O$ of and transfer it to the beaker. To dissolve the content, place it in the magnetic stirrer

Note: The solution should not give blue color by addition of acidified potassium iodide solution and starch.

b) Alkaline Iodide Sodium Azide Solution

To prepare this reagent we are going to mix three different chemicals

Dissolve either

- → 500 g of Sodium Hydroxide (or)
- → 700 g of Potassium Hydroxide
- → 135 g of Sodium Iodide (or)
- → 150 g of Potassium Iodide

To prepare this reagent, take 700 g of potassium hydroxide and add 150 g of potassium iodide and dissolve it in freshly boiled and cooled water, and make up to 1000 mL (One litre).

Dissolve 10 g of Sodium Azide (NaN_3) in 40 mL of distilled water and add this with constant stirring to the cool alkaline iodide solution prepared.

c) Sodium Thiosulphate stock solution

Weigh approximately 25 g of sodium thiosulphate $(Na_2S_2O_3.5H_2O)$ and dissolve it in boiled distilled water and make up to 1000 mL. Add 1 g of sodium hydroxide to preserve it.

d) Starch Indicator

Weigh approximately 2 g of starch and dissolve in 100 mL of hot distilled water. In case if you are going to preserve the starch indicator add 0.2 g of salicyclic acid as preservative.

e) Sulphuric Acid

f) Calcium Chloride solution

Weigh accurately 27.5 g of anhydrous calcium chloride and dissolve it in distilled water.

Take 100 mL standard measuring flask and place a funnel over it.

Transfer it to the 100 mL standard flask and make up to 100 mL using distilled water.

g) Magnesium Sulphate solution

Weigh accurately 22.5 g of magnesium sulphate and dissolve it in distilled water.

Take 100 mL standard measuring flask and place a funnel over it.

Transfer it to the 100 mL standard flask and make up to 100 mL using distilled water.

h) Ferric Chloride solution

Weigh accurately 0.15 g ferric chloride and dissolve it in distilled water.

Take 100 mL standard measuring flask and place a funnel over it.

Transfer it to the 100 mL standard flask and make up to 100 mL using distilled water.

i) Phosphate buffer solution

Weigh accurately 8.5g of Potassium Di Hydrogen Phosphate (KH_2PO_4) and dissolve it in distilled water.

Then add exactly 21.75 g of Di Potassium Hydrogen Phosphate (K_2HPO_4) and dissolve it.

To the same beaker 33.4 g of Di sodium hydrogen phosphate ($Na_2HPO_4.7H_2O$), is weighed and added.

Finally to the beaker containing all the salts, add accurately 1.7 g of Ammonium Chloride (NH_4Cl) and dissolve it.

Take 1000 mL standard measuring flask and place a funnel over it.

Transfer it to the 1000 mL standard flask and make up to 1000 mL using distilled water.

The pH should be 7.2 without further adjustment.

j) Dilution Water

High quality organic free water must be used for dilution purposes.

The required volume of water (five litres of organic free distilled water) is aerated with a supply of clean compressed air for at least 12 hours. Allow it to stabilize by incubating it at 20ºC for at least 4 hours.

For the test we have taken five litres of organic free aerated distilled water, hence add 5mL each of the nutrients.

- Add 5mL calcium chloride solution
- Add 5mL magnesium sulphate solution
- Add 5mL ferric chloride solution and

- Add 5mL phosphate buffer solution

This is the standard dilution water. Prepare dilution water 3 to 5 days before initiating BOD test to ensure that the BOD of the dilution water is less than 0.2 mg/L.

13.6.2 TESTING OF SAMPLE

- Take four 300 mL glass stoppered BOD bottles (two for the sample and two for the blank).
- Add 10 mL of the sample to each of the two BOD bottles and the fill the remaining quantity with the dilution water. i.e., we have diluted the sample 30 times.
- The remaining two BOD bottles are for blank, to these bottles add dilution water alone.
- After the addition immediately place the glass stopper over the BOD bottles and note down the numbers of the bottle for identification.
- Now preserve one blank solution bottle and one sample solution bottle in a BOD incubator at 20ºC for five days.
- The other two bottles (one blank and one sample) needs to be analysed immediately.

  Avoid any kind of bubbling and trapping of air bubbles. Remember – no bubbles!
- Add 2mL of manganese sulfate to the BOD bottle by inserting the calibrated pipette just below the surface of the liquid.
- Add 2 mL of alkali-iodide-azide reagent in the same manner.
- (The pipette should be dipped inside the sample while adding the above two reagents. If the reagent is added above the sample surface, you will introduce oxygen into the sample.)
- Allow it to settle for sufficient time in order to react completely with oxygen.
- When this floc has settled to the bottom, shake the contents thoroughly by turning it upside down.
- Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample.
- Carefully stopper and invert several times to dissolve the floc.

- Titration needs to be started immediately after the transfer of the contents to Erlenmeyer flask.
- Rinse the burette with sodium thiosulphate and then fill it with sodium thiosulphate. Fix the burette to the stand.
- Measure out 203 mL of the solution from the bottle and transfer to an Erlenmeyer flask.
- Titrate the solution with standard sodium thiosulphate solution until the yellow color of liberated Iodine is almost faded out. (Pale yellow color)

  Add 1 mL of starch solution.
- and continue the titration until the blue color disappears to colourless.
- Note down the volume of sodium thiosulphate solution added , which gives the D.O. in mg/L. Repeat the titration for concordant values.
- After five days, take out the bottles from the BOD incubator and analyse the sample and the blank for DO.
- Add 2mL of manganese sulfate to the BOD bottle by inserting the calibrated pipette just below the surface of the liquid.
- Add 2 mL of alkali-iodide-azide reagent in the same manner.
- If oxygen is present, a brownish-orange cloud of precipitate or floc will appear.
- Allow it to settle for sufficient time in order to react completely with oxygen.
- When this floc has settled to the bottom, shake the contents thoroughly by turning it upside down.
- Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample.
- Carefully stopper and invert several times to dissolve the floc.
- Titration needs to be started immediately after the transfer of the contents to Erlenmeyer flask.
- Rinse the burette with sodium thiosulphate and then fill it with sodium thiosulphate. Fix the burette to the stand.
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- Note down the volume of sodium thiosulphate solution added, which gives the D.O. in mg/L. Repeat the titration for concordant values.

13.7 CALCULATION

For determining the Biochemical Oxygen Demand in the given water sample, the readings should be tabulated.

13.7.1 TABLE

Trial No.	Day	Volume of Sample (mL)	Burette Reading (mL)		Volume of Titrant (mL) ($Na_2S_2O_3$ solution used)	Dissolved Oxygen (mg/L)
			Initial	Final		
Blank						
1.						
2.						
Blank						
1.						
2.						

Burette Solution: Sodium Thiosulphate

Pipette Solution: Sample

Indicator: Starch

End point : Disappearance of blue color

- For the calculation of initial DO, immediately after dilution the volume sample taken is 200 mL.
- For the blank titration the burette reading is 8.2 mL. The volume of titrant sodium thiosulphate is 8.2mL. The value of DO in mg/L is 8.2.
- For the first titration the burette reading is 7.9mL. The volume of titrant is 7.9 and the value of DO is 7.9 mg/L.
- For the second titration the burette reading is 7.9. The volume of titrant is 7.9 mL and the value of DO is 7.9 mg/L.

- For the calculation of DO at the end of five days the volume of sample taken is 200 mL. For the blank titration the value of burette reading is 8.0. The volume of titrant is 8.0mL and the DO is 8.0 mg/L.
- For the first titration the burette reading is 3.2. The volume of titrant is 3.2 and the DO value is 3.2 mg/L.
- For the second titration the burette reading is 3.2. The volume of titrant is 3.2 and the value of DO is 3.2 mg/L. We have achieved concordant values. So we can go for the calculations.

13.7.2 DATA SHEET

DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND
DATA SHEET

Date Tested : August 30, 2010

Tested By : CEM Class, Group A

Project Name : CEM, NITTTR Lab

Sample Number : BH1

Sample Location : Perungudi (Lat 12' 57" 31.74 & Long 80'14" 8.82)

Sample Description : Surface water

Trial No.	Day	Volume of Sample (mL)	Burette Reading (mL)		Volume of Titrant (mL)	Dissolved Oxygen (mg/L)
			Initial	Final		
Blank	0	200	0	8.2	8.2	8.2
3.	0	200	0	7.9	7.9	7.9
4.	0	200	0	7.9	7.9	7.9
Blank	5	200	0	8.0	8.0	8.0
1.	5	200	0	3.2	3.2	3.2
2.	5	200	0	3.2	3.2	3.2

Specimen Calculation:

Initial DO of the diluted sample, D_0 = 7.9 mL
DO at the end of 5 days for the diluted sample, D_5 = 3.2 mL
Blank correction = C_0 - C_5, BC = 0.2 mL
Initial DO of the blank, C_0 = 8.2 mL
DO at the end of 5 days for the blank, C5 = 8.0 mL

Biochemical Oxygen Demand $= \frac{\{DO - D5 - BC\} \times \text{Volume of the diluted sample}}{\text{Volume of sample taken}}$

Biochemical Oxygen Demand (mg/L) = (7.9 - 3.2 - 0.2) x 200 / 10
= 4.5 x 200 / 10
= 90 mg/L

13.8 INTERPRETATION OF RESULTS

The BOD of the given sample of water = **90 mg/L.**

13.9 INFERENCE

On the basis of the BOD values, the characteristics of the water and the biological activity of the incubated microflora can be determined. Effluent with high BOD levels is discharged into a stream or river; it will accelerate bacterial growth in the river and consume the oxygen levels in the river. The oxygen may diminish to levels that are lethal for most fish and many aquatic insects. As the river re-aerates due to atmospheric mixing and as algal photosynthesis adds oxygen to the water, the oxygen levels will slowly increase downstream. The biological capacity of a sewage treatment plant can be tested by comparing the BOD value of a known control solution with the BOD derived from the treatment plant.

BOD detects only the destructible proportion of organic substances and as a general principle is therefore lower than the COD value, which also includes inorganic materials and those materials which cannot be biologically, oxidized.

13.10 EVALUATION

1. Biochemical oxygen demand (BOD) is an important measure of
    a) the oxygen using potential of water and wastewater
    b) oxygen content of water and wastewater
    c) an organism's natural level of oxygen requirement
    d) a measure of the biological activity of water and wastewater

2. In BOD test, dilution water is aerated
    a) for supplementing air
    b) for cooling the sample
    c) for super saturation
    d) for diluting the sample

3. Which of the following is added as nutrient
    a) Calcium chloride
    b) Calcium sulphate
    c) Magnesium chloride
    d) Magnesium phosphate

4. Seeding is the process of addition of

a) seeds
b) live microbes
c) cold water
d) nutrients

5. After the incubation period of BOD which is 5 days at 20°C,

a) all the organic content would be exhausted.
b) all organisms present will die
c) practical convenience
d) all the nutrients would be exhausted.

6. In a treatment plant when the influent BOD is 245 mg/L and the effluent BOD is 22 mg/L, the percentage of BOD removed is

a) 19%
b) 91%
c) 9%
d) 86%

7. The reaction that occurs between iodine and sodium thiosulphate result in _____.

a) Sodium iodide
b) Disodium iodide
c) Disodium thioiodide
d) Sodium thio iodide

8. Manganous hydroxide takes up dissolved oxygen in molecular form to form Manganous oxide.

a) Manganous oxide
b) Manganous di oxide
c) Manganic di oxide
d) Manganic oxide

9. Sulphuric acid is added to

a) reduce tetravalent manganese to trivalent manganese
b) reduce tetravalent manganese to divalent manganese
c) reduce tetravalent manganese to manganese
d) make acidic pH

10. The increased level of BOD in water indicate that

a) it is not fit for potable use
b) it is fit for potable use
c) it tastes better
d) it smells pleasant

KEY TO ITEMS:

1) a
2) c
3) a
4) b
5) a
6) b
7) a
8) a
9) b
10) a