SEMESTER VI

16BTU612B ENVIRONMENTAL BIOTECHNOLOGY PRACTICAL 4H - 2C

Total hours/week: L:0 T:0 P:4 Marks: Internal: 40 External: 60 Total: 100

1. Calculation of Total Dissolved Solids (TDS) of water sample.
2. Calculation of BOD of water sample.
3. Calculation of COD of water sample.
4. Bacterial Examination of Water by MPN Method.

References

1. Santra, S.C. (2011). *Environmental Science* (3rd ed.). New Central Book Agency.
2. Pradipta Kumar Mohapatra, (2007). *Environmental Biotechnology*. I.K. International Publishing House.
3. Hans-Joachim Jordening,& Jesef Winter, (Eds.). (2005). *Environmental Biotechnology: Concepts and Applications*. Wiley-VCH.
4. Metcalf, & Eddy, (2003). *Waste Water Engineering: Treatment and Reuse* (4th ed.). Tata McGraw hill.
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SYLLABUS

Environmental Biotechnology

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2. Calculation of BOD of water sample.

3. Calculation of COD of water sample.

4. Bacterial Examination of Water by MPN Method.

Reference

Aneja, K.R. (2004). Experiments in Microbiology Plant Pathology and Biotechnology. New Delhi: New Age International.

Metcalf, L., & Eddy, R. (2005). Waste Water Engineering. New Delhi: Tata McGraw Hill.

Palvannan,T., Shanmugam, S., & Sathishkumar, T. (2005). Laboratory Manual on Biochemistry, Bioprocess and Microbiology. Chennai: SciTech Publications India Pvt. Ltd.

ENVIRONMENT BIOTECHNOLOGY PRACTICAL

1. Calculation of Total Dissolved Solids (TDS) of water sample.

Aim: To determine the total dissolved solids in the given water sample.

Principle: The term total dissolved solids refer to materials that are completely dissolved in water. These solids are filterable in nature. It is defined as residue upon evaporation of filterable sample. The term total suspended solids can be referred to materials which are not dissolved in water and are non filterable in nature. It is defined as residue upon evaporation of non filterable sample on a filter paper.

A well mixed sample is filtered through a standard glass fiber filter, and the filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 179-181°C. The increase in dish weight represents the total dissolved solids.

A well mixed sample is filtered through a weighed standard glass fiber filter and the residue retained on the filter is dried to a constant weight at 103-105°C. The increase n weight of the filter represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, the difference between the total solids and total dissolved solids may provide an estimate of the total suspended solids.

Materials required: Evaporating Dish, Water Bath, Oven, Desiccators, Balance

Procedure:

1. To measure total dissolved solids, take a clean porcelain dish which has been washed and dried in a hot air oven at 180(C for one hour.

2. Now weigh the empty evaporating dish in analytical balance. Let's denote the weight measured as W1.

3. Mix sample well and pour into a funnel with filter paper. Filter approximately 80 -100 mL of sample.

4. Using pipette transfer 75mL of unfiltered sample in the porcelain dish.

5. Switch on the oven and allowed to reach 105°C. Check and regulate oven and furnace temperatures frequently to maintain the desired temperature range.

6. Place it in the hot air oven and care should be taken to prevent splattering of sample during evaporation or boiling.

7. Dry the sample to get constant mass. Drying for long duration usually 1 to 2 hours is done to eliminate necessity of checking for constant mass.

8. Cool the container in a desiccator. Desiccators are designed to provide an environment of standard dryness. This is maintained by the desiccant found inside. Don't leave the lid off for prolonged periods or the desiccant will soon be exhausted. Keep desiccator cover greased with the appropriate type of lubricant in order to seal the desiccator and prevent moisture from entering the desiccator as the test glassware cools.

9. We should weigh the dish as soon as it has cooled to avoid absorption of moisture due to its hygroscopic nature. Samples need to be measured accurately, weighed carefully, and dried and cooled completely. Note the weight with residue as W2.

Calculation: Total Dissolved Solids (mg/L) TDS= W2 - W1

Result:

2. Calculation of BOD of water sample.

Aim: To determine biochemical oxygen demand in the given water sample

Principle: 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. 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). 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.

Materials Required:

- BOD Incubator
- Burette & Burette stand
- 300 mL glass stopper BOD bottles
- 500 mL conical flask
- Pipettes with elongated tips
- Pipette bulb

- 250 mL graduated cylinders
- Wash bottle

Chemicals

Calcium Chloride, Magnesium Sulphate, Ferric Chloride, Di Potassium Hydrogen Phosphate, Potassium Di Hydrogen Phosphate, Di sodium hydrogen phosphate, Ammonium Chloride, Manganous sulphate, Potassium hydroxide, Potassium iodide, Sodium azide, Concentrated sulfuric acid, Starch indicator, Sodium thiosulphate

Procedure:

• 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.

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•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

Calculation:

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

Biochemical Oxygen Demand = {D0− D5 − BC} x Volume of the dilutedsample / Volume of sample taken

Result:

4. Calculation of COD of water sample.

Aim: To determine chemical oxygen demand in the given water sample.

Principle: The chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of CODdetermine the amount of organic pollutants found in surface water (e.g. lakes and rivers), making COD a useful measure of water quality. It is expressed in milligrams per liter (mg/L), which indicates the mass of oxygen consumed per liter of solution. COD is the measurement of the amount of oxygen in water consumed for chemical oxidation of pollutants. COD determines the quantity of oxygen required to oxidize the organic matter in water or waste water sample, under specific conditions of oxidizing agent, temperature, and time. This method covers the determination of COD in ground and surface waters, domestic and industrial wastewaters. The applicable range is 3-900 mg/L.

The organic matter present in sample gets oxidized completely by potassium dichromate ($K_2Cr_2O_7$) in the presence of sulphuric acid (H_2SO_4), silver sulphate ($AgSO_4$) and mercury sulphate ($HgSO_4$) to produce CO_2 and H_2O. The sample is refluxed with a known amount of potassium dichromate ($K_2Cr_2O_7$) in the sulphuric acid medium and the excess potassium dichromate ($K_2Cr_2O_7$) is determined by titration against ferrous ammonium sulphate, using ferroin as an indicator. The dichromate consumed by the sample is equivalent to the amount of O_2 required to oxidize the organic matter.

Materials Required:

- COD Digester
- Burette & Burette stand
- COD Vials with stand
- 250 mL conical flask (Erlenmeyer Flask)

- Pipettes
- Pipette bulb
- Tissue papers
- Wash Bottle

Chemicals Required:

- Potassium dichromate
- Sulfuric acid
- Ferrous ammonium sulphate
- Silver sulphate
- Mercury sulphate
- Ferroin indicator
- Organic free distilled water

Procedure:

a) Standard Potassium Dichromate Reagent - Digestion Solution

- Weigh accurately 4.913 g of potassium dichromate, previously dried at 103ºC for 2 - 4 hours and transfer it to a beaker.
- Weigh exactly 33.3g of mercuric sulphate and add to the same beaker.
- Measure accurately 167 mL of concentrated sulphuric acid using clean dry measuring cylinder and transfer it to the beaker. Dissolve the contents and cool to room temperature. (If not dissolved keep it over night).

- Take 1000 mL standard measuring flask and place a funnel over it.
- Carefully transfer the contents to the 1000 mL standard flask and make up to 1000 mL using distilled water.
- This is the standard potassium dichromate solution to be used for digestion.

b) Sulphuric Acid Reagent - Catalyst Solution

- Weigh accurately 5.5 g silver sulphate crystals to a dry clean 1000 mL beaker.
- To this carefully add about 500 mL of concentrated sulphuric acid and allow to stand for 24 hours (so that the silver sulphate crystals dissolve completely).

c) Standard Ferrous Ammonium Sulphate solution

- Weigh accurately 39.2g of ferrous ammonium sulphate crystals and dissolve it in distilled water.
- Take 1000 mL standard measuring flask and place a funnel over it.
- Carefully transfer the contents to the 1000 mL standard flask and make up to 1000 mL mark using distilled water.

Testing of Sample

1. Take three COD vials with stopper (two for the sample and one for the blank).

2. Add 2.5 mL of the sample to each of the two COD vials and the remaining COD vial is for blank; to this COD vial add distilled water.

3. Add 1.5 mL of potassium dichromate reagent - digestion solution to each of the three COD vials.

4. Add 3.5 mL of sulphuric acid reagent - catalyst solution in the same manner.

5. Cap tubes tightly. Switch on the COD Digester and fix the temperature at 150º C and set the time at 2 hours.

6. Place the COD vials into a block digester at 150°C and heat for two hours.

7. The digester automatically switches off. Then remove the vials and allow it to cool to the room temperature.

8. Meanwhile, get ready with the burette for the titration.

9. Fill the burette with the ferrous ammonium sulphate solution, adjust to zero and fix the burette to the stand.

10. Transfer the contents of the blank vial to conical flask.

11. Add few drops of ferroin indicator. The solution becomes bluish green in colour.

12. Titrate it with the ferrous ammonium sulphate taken in the burette.

13. End point of the titration is the appearance of the reddish brown colour.

14. Note down the volume of ferrous ammonium sulphate solution added for the blank (A). Transfer the contents of the sample vial to conical flask.

15. Add few drops of ferroin indicator. The solution becomes green in colour.

16. Titrate it with the ferrous ammonium sulphate taken in the burette.

17. End point of the titration is the appearance of the reddish brown colour.

18. Note down the volume of ferrous ammonium sulphate solution added for the sample (B).

Calculation

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

Volume of Ferrous Ammonium sulphate for blank (A) = 14.1 mL

Volume of Ferrous Ammonium sulphate for Sample (B) = 13.2 mL

Normality of Ferrous Ammonium sulphate=0.1 N

Volume of Sample=2.5 mL

Chemical Oxygen Demand = (A - B * N * 8 * 1000)/ Volume of sample taken.

Result:

5. Bacterial Examination of Water by MPN Method.

Aim: The Aim of this study was to determine heavy metals levels (i.e. Cr and Fe), in different samples (i.e. surface water, and therapeutic mud) collected from different locations.

Principle

The term "Trace Metals" refers to metals which may be present in foods in amounts well below 50 mg / kg and which have some toxicological or nutritional significance While some inorganic elements such as, sodium, potassium calcium, phosphorous are essential for man, elements like lead, cadmium, mercury, arsenic are found to cause deleterious effects even in low levels of 10 – 50 mg / Kg. Although iron, copper, zinc, etc., are found to be necessary in certain quantities in foods, the same elements can cause ill effects when consumed at higher levels. Hence, determination of both major and trace levels of metal contents in food is important for both food safety and nutritional considerations. Steps involved in Assay of Metals: There are four major steps involved in the analysis of foods for the metal contents, viz. (a) Obtaining a representative sample from the bulk received for testing. (b) Destruction of organic matter. (c) Separation and concentration of the element of interest and (d) Determination

Apparatus and materials

Beakers - 250 mL, or equivalent. Temperature sensing device, e.g. thermometer, thermistor, thermocouple, or equivalent, capable of measuring temperatures between 0 and 150EC. Filter paper - Whatman No. 41, or equivalent. Funnels - polypropylene, or equivalent. Heating device, e.g. hot plate, heating block, microwave or equivalent. Volumetric flasks, of suitable precision and accuracy. Volumetric pipettes, of suitable precision and accuracy. Stirring device, e.g. magnetic stirrer, glass rod or equivalent. NOTE: All glassware should be acid washed.

Sampling:

The object of this step is to obtain a small and representative portion from the large sample in such a way that any subsequent test on the sample will give a reproducible value. For fresh foods, the homogenization process is like macerating in a blender whereas dry products are

normally ground mechanically and then mixed and the powder is sieved before analysis. Contamination during this step can be avoided with the use of stainless steel equipment.

Preparation of Sample:

Digestion by Microwave Method:

Weigh accurately about 25 g of well homogenised sample into a clean silica dish. Add 25 ml of 20% sulphuric acid (b). Mix thoroughly with a glass stirring rod ensuring all sample material is wetted by the acid. Rinse stirring rod with water into silica dish. Dry the contents of the dish thoroughly on a steam bath or in an oven around 110ºC. When the sample is thoroughly dry, heat the contents of the dish with a soft flame (such as that of a Bunsen burner) until all volatile or readily combustible matter has been removed. Transfer the dish to a furnace set at 250ºC. Slowly raise temperature to 500 ºC. Ash at this temperature for about 6 to 8 hours. Remove the dish and cool. Ash should now be white or brownish red and essentially be carbon free. If ash contains carbon particles, wash down sides of dish with water and add 2 ml of HNO3 and mix well. Dry thoroughly on hot plate. Return dish to furnace at 500ºC and ash for 30 minutes. Repeat nitric acid treatment using 1 ml increments of HNO3 until white/brownish red, carbon free ash is obtained. When clean ash is obtained, remove the dish from furnace, cool and add 1ml HNO3 and 10 ml of water. Heat on hot plate till sample ash is dissolved. Quantitatively transfer the contents of the dish to a 50 ml volumetric flask, heat the dish with 10 ml of HCl (1+1) and transfer the solution again to the same volumetric flask to volume with water.

Prepare sample blank solution by following the same procedure as described for sample. Use same quantities of reagents including water for both sample and blank. Subject both sample and sample blank to identical treatment (even the length of time kept in furnace etc.)

NOTE: calibrate AAS with copper solution (NIST tractable) before use, for absorption value (pre defined).

Determination: Atomic absorption Spectrophotometry:- iron and Chromium in samples can be determined by flame AAS (1) Set the instrument as per the previously established optimum

conditions /as per the guide lines given in the Instruction Manual (provided along with the instrument). The standard conditions for Atomic absorption spectrophotometer are given below. (2)Determine absorbance of sample solution(s) and blank. (2)Calculate the heavy metal content from standard curve.

Result: