## **Biochemical Oxygen Demand**

A bioassay test, involving measurement of oxygen consumed by micro-organisms while stabilizing biologically decomposable organic matter under aerobic conditions

Need

To determine the pollution load of waste water
The degree of pollution in water sources
Self purification capacity of sources
Designing of treatment facilities
Efficiency of waste water treatment methods

## Methodology

## Principle

The BOD test is based upon determinations of dissolved oxygen

 $\succ$  It can be measured directly

 $\succ$  In general, a dilution procedure is applied.

## Procedure

### Preparation of dilution water

- Aerate the required volume of D.W. by bubbling compressed air for 1-2 days to attain D.O. saturation
- Add 1 ml each per litre of dilution water Phosphate buffer Magnesium sulphate Calcium chloride Ferric chloride
- ≻ Mix well
- In case, waste not expected sufficient bacterial population, add seed (2 ml settle sewage / litre of dilution water)

## Determination of D.O.

- i) Samples and ii) Blank, on initial and after 5 days
- 2 ml MnSO<sub>4</sub> + 2 ml Alkali-iodide-azide+stopper immediately
- $\succ$  Mix well + allow the ppt. to settle
- > Add 2 ml concentrated H<sub>2</sub> SO<sub>4</sub> + mix well till ppt. dissolve
- Take 203 ml (correspond to 200 ml) sample in a conical flask
- Titrate against sodium thiosulphate (0.025 N) till pale yellow colour + starch solution + blue colour + titrate till colourless

### Observations

 $D_0 = D.O.$  in sample on 0<sup>th</sup> day  $D_1 = D.O.$  in sample on 5<sup>th</sup> day  $C_0 = D.O.$  in Blank on 0<sup>th</sup> day  $C_1 = D.O.$  in Blank on 5<sup>th</sup> day

 $C_0 - C_1 = D.O.$  depletion in dilution water alone

 $D_0 - D_1 = D.O.$  depletion in sample + dilution water

 $(\overline{D}_0 - \overline{D}_1) - (\overline{C}_0 - \overline{C}_1) = \overline{D}.O.$  depletion due to microbes

## Calculation

1 ml of 0.025 N sodium thiosulphate = 0.2 mg of Oxygen D.O. in mg/l =  $(0.2 \times 1000) \times ml$  of thiosulphate 200

B.O.D. in mg/l  $(D_0-D_1) - (C_0-C_1)$  mg X Decimal fraction of sample used

Results B.O.D. 5 days at  $20^{\circ}$  C = mg/l

## Interferences

- Ferrous ion
- $\succ$  Ferric ion
- > Nitrate
- Microbial mass
- High suspended solids
  - ✤ Lack of nutrients in dilution water
  - ✤ Lack of acclimated seed organisms
  - Presence of heavy metals
  - Presence of toxic materials

# **Bacteriological Analysis**

## Bacteria Single cell microscopic organisms lacking chlorophyll Coliform group

#### Contamination

- Insanitary condition of surrounding area
- Unhygienic practices
- Discharge/seepage of sewage and domestic wastewater

#### Need

- Impact on water quality
- Potability for human consumption
- To prevent water-borne diseases
- To assess the quality of raw and treated water
- Specially to detect Faecal Contamination

#### Bacteriological analysis : mainly includes estimation of

- Total coliforms
- Faecal coliforms

# Methodology

Approved techniques generally used as per "Standard Methods for the examination of water and wastewater"

- .Membrane Filter (MF)
- Multiple Tube Dilution (MTD)

### **MF-technique**

### **Principle**

Biochemical reactions are used to detect the various groups of micro-organisms

#### **MF-technique - Merits**

- Results in 24 hours (MTD 48 to 96 hours)
- Larger volume of samples can be tested (MTD less volume)
- Results with greater precision (MTD MPN)
- Require less laboratory space (MTD More space)
- Easy processing (MTD Tedious)
- Useful during normal and emergencies (MTD Difficult in emergencies)

#### Limitations : Samples with more turbidity

# Colour

- Coloured water is not acceptable for drinking (Aesthetic as well as toxicity reasons)
- Industrial wastewater require colour removal before discharge into water courses

### Definition

- The term colour means true colour that is the colour of water from which turbidity has been removed. True colour of water is due to dissolved material
- Apparent colour is due to suspended matter as well as due to substances on solution removed by filtration

#### **Unit for Measurement of colour**

• Unit for colour measurement is based on platinum cobalt scale



#### **Methods for Colour Measurement**

#### **Visual Comparison Method**

- Colour of the sample is determined by visual comparison with known concentration of coloured colutions prepared by diluting stock platinum cobalt solution
- OR properly calibrated glass coloured disk is used for comparison
- This method is useful for potable water and water in which colour is due to naturally occuring materials
- This method is not applicable to most highly coloured industrial wastewater

#### Spectrophotometric Method

- This method is applicable to potable and waste both domestic and industrial
- In this method light absorbed or transmitted is measured at dominant wavelength of a particular hue of sample
- Spectrophotometer should have an effective operating range from 400 to 700 nm before measurement remove turbidity either by filtration or by centrifuging
- Colour hues for dominant wavelengths ranges are