# ESTIMATION OF MICROBIAL CONTAMINATION IN LAKE WATER

*A PRECURSOR TOWARDS INCREASED BIOLOGICAL DEMAND FOR OXYGEN*

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***ABSTRACT :***

With the pollution levels on their peak and a drastic increase in water body pollution, the current scenario is taking a toll on the lives of aquatic life forms- both marine as well as fresh water. The major cause of concern is the Biological Demand for Oxygen abbreviated as BOD which refers to the amount of oxygen present per millilitre of water. BOD estimates the utilization of oxygen by the micro organisms present in the water body to break down or degrade complex organic substances into simpler compounds which is indirectly used for fulfilling their energy requirements in order to carry out various metabolic processes. As a result, it deprives the aquatic organisms of their required amount of oxygen, on in other words their oxygen supply is "cut off" and they eventually die. The major contributors of water pollution are:

- Industrial discharge (effluents)
- Domestic sewage
- Waste from religious gatherings
- Animal and human waste matter
- Agricultural and livestock waste
- Debris from natural calamities

Recent studies have shown a 60-70% increase in water pollution with 45-50% attributing towards microbial contamination. Different projects have been undertaken to reduce waste water pollution and replenish the natural ecosystem of water bodies. As individuals also, measures can be adopted to curb water pollution and we can do out bit to save whatever remaining percentage potable water we have on out planet. The aim of this report is to estimate the amount of oxygen present in the water sample isolated from Palanahalli Lake (Bangalore) and therefore, determine the concentration of microbial contamination present in the selected sample. The report also focuses on the different measures that can be adopted to reduce water body adulteration.

## INTRODUCTION

## OBJECTIVE OF THE STUDY

The research had been done keeping the following key points in mind:

- Collection of ample quantity of sample
- Analysis of the extent of contamination
- Isolation of sufficient microbial colonies
- Identification of colonies
- Determination of BOD (Biological Demand for Oxygen)
- Staining procedures for the identification of the type of bacterial species

## CONCEPT OF BOD

BOD or BIOLOGICAL DEMAND FOR OXYGEN is defined as the amount of oxygen present per millilitre of water.
Because of increased concentration of organic pollutants in the water bodies, bacteria and other micro organisms utilize the oxygen dissolved in the water to break down these organic substances into simpler compounds.
As a result, the aquatic life forms and other marine organisms are deprived of their required supply of oxygen.
Thus, the amount of BOD is directly proportional to the concentration of organic contaminants present in the water body.
Hence, the aim of this study is to show the extent of pollutants that have contaminated the water bodies with an attempt to re mediate them.

## METHODOLOGY

Appropriate amount of lake water sample was collected. This was followed by preparation of specific chemical reagents. The collected sample was subjected to tit-ration in order to determine the amount of dissolved oxygen present in it. The sample was incubated which was followed by another round of tit-ration and the BOD value (post incubation) was thus, determined. The sample was also used for the preparation of colony plates. After estimating the number of colonies, Gram Staining was carried out in order to determine the type of bacterial colony and segregate them on the basis of their cell wall composition.

## REVIEW OF LITERATURE

**Kim et al., 2003** reported that high concentrations of organic matter (BOD, COD) and high microbial concentrations (as indicated by total coli forms) were observed in groundwater affected by livestock. He also inferred that this was due to the faster loss of easily biodegradable organic matter compared with non-biodegradable organic matter.

**Emongor et al., 2005** in his study concluded that brewery, chemical and paints and food and beverage industries are the major sources of high COD, BOD and suspended solids discharge. COD, BOD, suspended solids and heavy metal levels should be monitored strictly in order to prevent environmental pollution and reduce health hazards caused by pollutants

## PROCEDURE OF EXPERIMENT

**1.Preparation of chemical reagents** (all the chemicals were dissolved in distilled water)-

- Sodium azide solution (0.1 g in 10 ml)
- Magnesium sulphate solution (4.8 g in 10 ml)
- Starch solution (indicator) (0.5 g in 50ml)
- Sodium thiosulphate solution (0.6 g in 100ml)

**2.Titration of water sample prior to incubation**

- 250 ml of water sample was taken in a BOD bottle. To this 2ml of $MnSo_4$ solution was added followed by alkaline azide solution.
- The bottle was shaken for 3-4 times and the brown precipitate thus formed, was allowed to settle.
- 2ml of concentrated $H_2SO_4$ was added and the precipitate was allowed to dissolve.
- Titration of the sample was carried against $Na_2S_2O_3$ to obtain concordant values and the end point was determined using 1% starch solution. The end point being blue to colourless.

**OBSERVATION: Prior to Incubation**

| Serial Number | Initial burette reading (ml) | Final burette reading (ml) | Volume of $Na_2S_2O_3$ run down (ml) |
|---|---|---|---|
| 1 | 0.0 | 0.5 | 0.5 |
| 2 | 0.5 | 1.0 | 0.5 |
| 3 | 1.0 | 1.5 | 0.5 |
| 4 | 1.5 | 2.0 | 0.5 |
| 5 | 2.0 | 2.4 | 0.4 |

## CALCULATION I

$$\text{Dissolved Oxygen} = \frac{(8 \times 1000 \times N)\, v}{V}$$

$$= \{(8 \times 1000 \times 0.025) 0.5\}/50$$

$$= 2.0 \text{ mg/L}$$

Therefore, D1=2.0mg/L where, D1 = amount of oxygen present prior to incubation
The sample was incubated in the dark for 3 days (72 hours) and titration was carried out in the similar manner.

**OBSERVATION: Post Incubation**

| Serial Number | Initial burette reading (ml) | Final burette reading (ml) | Volume of $Na_2S_2O_3$ run down (ml) |
|---|---|---|---|
| 1 | 0.0 | 0.7 | 0.7 |
| 2 | 0.7 | 1.4 | 0.7 |
| 3 | 1.4 | 2.1 | 0.7 |
| 4 | 2.1 | 2.7 | 0.6 |
| 5 | 2.7 | 3.2 | 0.5 |

**CALCULATION II**

Dissolved Oxygen= $\frac{(8x1000xN)\ v}{V}$

$=\{(8x1000x0.025)0.7\}/50$

$=2.8mg/L$

Therefore, D2=2.8mg/L

BOD= D2-D1 = 2.8 - 2.0 = 0.8 mg/L

Where, D1= amount of dissolved oxygen prior to incubation

D2= amount of dissolved oxygen post incubation

**RESULT**

The BOD value for the selected sample of lake water was 0.8mg/L

**3. Preparation of colony plates**

- Nutrient agar media was prepared.
- The pH of the media was maintained at 7.2.
- The culture media was sterilized in an autoclave and the liquid media was then transferred to petri plates.
- Using streak plate method, microbial colonies were made.
- The petri plates were incubated at 37°C.

**Observation**

Different patterns of microbial colonies were observed after an incubation period of 24 hours. The colonies were counted and estimated using a colony counter and expressed as CFU (Coli form unit) which showed that the colony was dominated by bacterial growth.

**4. Gram Staining**

- From the petri plates a loop full of bacterial culture was isolated which was streaked onto a slide.
- The smear was heat fixed with flame and was stained with Crystal Violet stain.
- This was followed by washing and staining with Gram's Iodine.
- The stain was drained off and the smear was stained with Safranin dye.
- The slides were observed under a compound microscope.

**Observation**

Purple and pink coloured colonies of bacteria were observed. However, the population was dominated by Gram positive bacterial cells that retained the Gram stain and appeared purple while, cells which retained the counter stain Safranin appeared pink (Gram Negative).

**CONCLUSION**

We observed increased BOD and more number of bacterial colonies in the sample, which indicates the presence of organic matter in the lake which contribute towards lake water contamination.

This suggested that the pollution has increased at an alarming rate for which serious measures have to be taken to preserve the water bodies.

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