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Chapter 20

# Estimation of biochemical oxygen demand (BOD): Principles, methodology, and water quality assessment

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## 1. Introduction:

Biochemical Oxygen Demand (BOD) serves as a vital gauge for evaluating organic pollution levels in aquatic ecosystems (Xu et al 2020; Tripathi et al., 2013). It quantifies the oxygen consumed by microorganisms while breaking down organic substances within water. BOD stands as a pivotal marker in water quality assessment, prominently employed in both environmental surveillance and wastewater treatment strategies (Metcalf and Eddy, 2013; Tripathi et al., 2018).

### 1.1. BOD Mechanism:

Upon the introduction of organic materials like sewage, industrial discharges, or agricultural residues into water sources, microbial communities, including bacteria and fungi, initiate their metabolic breakdown. This decomposition process, integral to their life cycle, entails oxygen consumption, thereby diminishing the dissolved oxygen (DO) levels within the water column. The magnitude of organic pollutants directly correlates with the oxygen demands of microorganisms, thus amplifying the BOD value (Sawyer et al., 2003).

### 1.2. Importance of BOD:

1. **Water Quality Assessment:** Vital for evaluating aquatic ecosystem health, elevated BOD levels signify poor water quality, causing oxygen depletion and endangering aquatic life.
2. **Wastewater Management:** BOD testing is integral to gauge wastewater treatment efficiency. Monitoring BOD in influent and effluent aids in process optimization and ensures compliance with regulatory standards.
3. **Environmental Impact:** High BOD levels trigger hypoxic or anoxic conditions, harming aquatic ecosystems. Monitoring BOD helps pinpoint pollution sources and implement mitigation measures.

4. **Regulatory Compliance:** Numerous environmental regulations stipulate BOD limits to safeguard aquatic habitats and human health, necessitating regular monitoring and reporting by industries and municipalities.

## 2. Principle of Biochemical Oxygen Demand (BOD) Testing:

The test methodology involves enclosing a water sample in an airtight container and situating it in a controlled environment at a consistent temperature for a five-day period. Measurements of dissolved oxygen (DO) levels are taken both at the beginning and end of this incubation interval. The test is standardized to operate at a temperature of 20°C. Taking the original DO concentration less the end DO concentration yields the BOD value. The first DO measurement takes place right away following sample dilution to make sure that any oxygen used later in the testing phase is taken into account when computing the BOD.

## 3. Calculation of BOD:

The calculation of BOD follows a specific formula, which encapsulates the amount of oxygen consumed during this process.

BOD = (DO1 - DO2) x (Dilution Factor) / (Volume of Sample)

Where:

BOD = Biochemical Oxygen Demand in milligrams per litre (mg/L).

DO1 = Initial concentration of Dissolved Oxygen in mg/L.

DO2 = Final concentration of Dissolved Oxygen after the test period in mg/L.

**Dilution Factor:** The Dilution Factor represents the ratio of the original sample volume to the volume of the sample after dilution, used to adjust for volume reduction during dilution. This factor plays a crucial role in the BOD formula when dilution is necessary. The Volume of Sample refers to the initial volume of the undiluted water sample, typically measured in milliliters (mL). In the process of determining BOD, the initial dissolved oxygen concentration (DO1) is subtracted from the final dissolved oxygen concentration (DO2). The resulting difference is then multiplied by the dilution factor and divided by the volume of the original sample. The outcome is the BOD expressed in milligrams per liter (mg/L), which measures the degree of pollution in the water sample and shows how much oxygen microbes are consuming.

The BOD value must be reported in mg/L as the last stage of the test process. It shows how much oxygen bacteria consumed during the test. This amount shows how much organic contamination the water sample has.

## 4. Materials required:

1. BOD Test Apparatus
2. BOD incubator:

3. Burette and stand
4. 300ml BOD bottle
5. DO Meter
6. Wash bottle
7. Conical flask
8. Measuring cylinder
9. Pipette with elongated tip
10. Gloves
11. seal starch:
12. Alkaline-iodide-azide solution
13. Manganese sulphate
14. Con. Sulphuric acid
15. Starch solution
16. 0.025N sodium thiosulphate

## 5. Procedure:

Determining the Biological Oxygen Demand (BOD) of water involves a standardised procedure that includes several steps. To get an insight into how is BOD measured, check the procedure given below:

### 5.1. Neutralization of Sample:

1. Pour 50 milliliters of the water sample into a 100-milliliter beaker.
2. Take a calibrated pH meter reading on the sample.
3. Using 1N sulfuric acid, bring the pH to 7.00 ± 0.2 if it is more than 7. Apply 1N sodium hydroxide to correct if the pH is less than 7.00.
4. Record the amount of sodium hydroxide or sulfuric acid needed to raise the pH of the 50 ml sample to 7.00 ± 0.2.
5. Determine how much sodium hydroxide or sulfuric acid will neutralize the whole 1000 ml sample.
6. To totally neutralize the sample, add the amount of sulfuric acid or sodium hydroxide that was estimated.

### 5.2. Removal of Chlorine Content:

1. Pour a 50 ml water sample into a conical flask and shake.
2. Pour into the flask 2.5 ml of a 10% w/v potassium iodide solution and 2.5 ml of 50% diluted acetic acid.
3. Stir in 1 milliliter of starch indicator and titrate with a sodium sulphite solution 0.025N.
4. As described in the section on neutralization of samples, note the titration volume and determine the amount needed to neutralize a 1000 ml sample.

5. To neutralize the chlorine, add the determined volume of sodium sulfite solution to the sample.

### 5.3. Preparation of Phosphate Buffer Solution:

1. Dissolve in 500 millilitres of pure water: 8.5 grams of potassium dihydrogen phosphate ($KH_2PO_4$), 21.75 grams of dipotassium hydrogen phosphate ($K_2HPO_4$), 33.4 grams of disodium hydrogen phosphate ($Na_2HPO_4.7H_2O$), and 1.7 grams of ammonium chloride ($NH_4Cl$).
2. Adjust the volume of the solution to 1 liter by diluting it with additional distilled water

### 5.4. Preparation of Alkali-Iodide-Azide Reagent:

1. To make a 1000 milliliter solution, dissolve 500 grammes of sodium hydroxide (NaOH) and 135 grams of sodium iodide (NaI) in distilled water.
2. Dissolve ten grams of sodium azide in the above solution.

### 5.5. Preparation of Dilution Water:

1. Take five liters of double-distilled water and, for a minimum of twelve hours, air-condition it with clean compressed air.
2. Give the water six hours or more to stabilise at 20°C.
3. Add 5 milliliters each of the magnesium sulphate solution (22.5%), ferric chloride (0.15% w/v) and calcium carbonate solutions (27.5% w/v).
4. Add five milliliters of the phosphate buffer solution and mix thoroughly.
5. Let the mixture stand for two hours.

### 5.6. Measurement procedure:

1. To two of the 300 ml BOD bottles, add 10 ml of the water sample; fill the empty space with dilution water.
2. Pour dilution water for the blank sample into the remaining two BOD bottles.
3. Seal the bottles straight away to ensure no air bubbles remain inside.
4. Four days at 20°C should be spent incubating one sample and one blank bottle.
5. Analyze the one sample and one blank vial of dissolved oxygen (DO) right away
6. Examine for DO the bottles that have been incubated for five days.

### 5.7. Dissolved Oxygen (DO) measurement:

1. Dispense 2 ml of a 36.4% manganous sulfate ($MnSO_4.H_2O$) solution into the water sample, ensuring the pipette tip is submerged to allow oxygen entry through solution droplets.
2. Employ the same method to introduce 2 ml of the alkali-iodide-azide reagent into the sample.

3. Permit the solutions to interact with the dissolved oxygen present in the sample.
4. To help precipitates dissolve, add 2 millilitres of concentrated sulfuric acid close to the sample's surface after it has settled.
5. Thoroughly blend to dissolve any remaining precipitates.
6. Transfer 203 ml of the BOD sample to an Erlenmeyer flask.
7. Conduct prompt titration using 0.025N Sodium Thiosulfate solution alongside a Starch indicator until the blue coloration dissipates, and record the burette reading.
8. Proceed similarly to find the burette reading for the blank sample.

## 6. Precautions:

1. Handle water samples carefully to prevent introduction of air bubbles that could affect results.
2. Keep samples at a consistent temperature throughout the incubation period.
3. Use appropriate volumes of seed inoculum to ensure accurate measurements.
4. Calibrate all instruments, such as dissolved oxygen probes, before use.
5. Perform blank tests using sterile water to account for any background BOD.
6. Use a sufficient number of replicates to ensure statistical reliability.
7. Record measurements consistently and accurately throughout the incubation period.
8. Avoid exposure of samples to direct sunlight or heat that could alter BOD levels.

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