

Estimation of dissolved oxygen

AIM: To estimate the amount of dissolved oxygen (DO) in a water sample using the Winkler method.

PRINCIPLE: The Winkler method for estimating dissolved oxygen involves a series of chemical reactions that produce an iodine equivalent to the dissolved oxygen present in the sample. The dissolved oxygen is fixed by adding manganese sulfate (MnSO_4) and alkaline potassium iodide (KI) to the water sample. In the presence of oxygen, manganese(II) ions are oxidized to manganese(IV) oxide. This manganese(IV) oxide then reacts with iodide ions (I^-) in an acidic medium to release iodine (I_2), which is titrated with a sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) solution to determine the concentration of dissolved oxygen.

REAGENTS:

1. Manganese sulfate solution (MnSO_4)
2. Alkaline potassium iodide solution (KI + NaOH)
3. Concentrated sulfuric acid (H_2SO_4)
4. Sodium thiosulfate solution ($\text{Na}_2\text{S}_2\text{O}_3$, 0.025 M)
5. Starch solution (as an indicator)
6. Distilled water

PROCEDURE:

1. Collection of Water Sample:

- Collect the water sample in a 300 mL BOD (Biochemical Oxygen Demand) bottle, ensuring no air bubbles are present.

2. Fixation of Dissolved Oxygen:

- Add 2 mL of manganese sulfate solution to the bottle.
- Add 2 mL of alkaline potassium iodide solution immediately.
- Stopper the bottle carefully to prevent air from entering and mix by inverting the bottle several times.
- A brown precipitate of manganese(IV) oxide will form if oxygen is present.

3. Acidification:

- Add 2 mL of concentrated sulfuric acid to the bottle.

- Replace the stopper and mix thoroughly until the precipitate dissolves, resulting in the formation of iodine.

4. Titration:

- Take 200 mL of the treated water sample into a conical flask.
- Titrate the sample with 0.025 M sodium thiosulfate solution until the yellow-brown color of iodine starts to fade.
- Add a few drops of starch solution, which will turn the solution blue.
- Continue titrating until the blue color disappears, indicating the endpoint.

5. Calculation:

- Record the volume of sodium thiosulfate solution used.
- Calculate the concentration of dissolved oxygen using the formula:

$$\text{Dissolved Oxygen (mg/L)} = \frac{(V_1 - V_2) \times N \times 8000}{V}$$

where:

- V_1 = Volume of sodium thiosulfate used for the titration of the sample (mL)
- V_2 = Volume of sodium thiosulfate used for the titration of the blank (mL) (if any)
- N = Normality of sodium thiosulfate solution
- V = Volume of the water sample taken for titration (mL)

RESULT: The calculated value represents the amount of dissolved oxygen present in the water sample in mg/L. This value indicates the oxygen available in the water, which is crucial for aquatic life. A high level of dissolved oxygen typically signifies good water quality, whereas low levels can indicate pollution or high biological activity consuming oxygen.

Estimation of salinity in water samples

Aim : To estimate the salinity of water samples using a titration method with silver nitrate.

Principle : Salinity in water samples can be estimated by determining the concentration of chloride ions (Cl^-). Chloride ions react with silver nitrate (AgNO_3) to form a precipitate of silver chloride (AgCl). The endpoint of the titration is indicated using potassium chromate (K_2CrO_4) as an indicator, which forms a red precipitate of silver chromate (Ag_2CrO_4) when all chloride ions have reacted.

Reagents

1. Silver nitrate solution (0.1 M)
2. Potassium chromate indicator solution (5%)
3. Distilled water
4. Water samples
5. Nitric acid (1 M) – optional, for pH adjustment if needed

Apparatus

1. Burette
2. Pipette
3. Conical flask
4. Beaker
5. White tile (for better visibility of color change)
6. Glass rod (for stirring)

Procedure

1. **Preparation of the Silver Nitrate Solution:**
 - Dissolve the required amount of silver nitrate in distilled water to prepare a 0.1 M solution.
2. **Preparation of the Potassium Chromate Indicator:**
 - Dissolve 5 grams of potassium chromate in 100 mL of distilled water to prepare a 5% solution.
3. **Sample Preparation:**
 - If the water sample is turbid, filter it to remove any suspended particles.
 - If necessary, adjust the pH of the water sample to around 7 using nitric acid.
4. **Titration:**
 - Pipette 50 mL of the water sample into a conical flask.
 - Add a few drops of potassium chromate indicator solution to the flask. The solution will turn yellow.
 - Fill the burette with the 0.1 M silver nitrate solution.

- Titrate the water sample with the silver nitrate solution, adding it slowly while continuously swirling the flask.
- Continue adding silver nitrate until a red precipitate (silver chromate) starts to form, indicating the endpoint of the titration.

5. Calculation:

Note the volume of silver nitrate solution used to reach the endpoint.

Use the formula to calculate the concentration of chloride ions:

$$\text{Cl}^- \text{ (MG/L)} = \frac{\text{Volume of water sample (L)} \times \text{Volume of AgNO}_3 \text{ (mL)} \times 35.45 \times 0.1 \text{ M}}{\text{Volume of water sample (L)}}$$

Convert the concentration of chloride ions to salinity (parts per thousand, ppt):

$$\text{Salinity (ppt)} = \text{Cl}^- \text{ (mg/L)} \times 0.001806$$

Results

Record the volume of silver nitrate solution used for each water sample. Calculate the concentration of chloride ions and the salinity for each sample. Present the results in a tabular form:

Sample ID	Volume of AgNO ₃ (mL)	Cl ⁻ Concentration (mg/L)	Salinity (ppt)
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Analyze the results to determine the salinity levels of the different water samples. Compare the values to standard salinity levels to assess the quality and type of water (e.g., freshwater, brackish water, seawater).

Estimation of Chemical Oxygen Demand (COD)

Aim

To estimate the Chemical Oxygen Demand (COD) of a water sample using the dichromate method.

Principle

Chemical Oxygen Demand (COD) is a measure of the amount of oxygen required to oxidize organic and inorganic matter in water. In this method, a known amount of potassium dichromate ($K_2Cr_2O_7$) is added to the water sample in an acidic medium, and the sample is then heated. The dichromate oxidizes the organic matter in the sample, and the remaining dichromate is titrated with a ferrous ammonium sulfate (FAS) solution. The amount of dichromate consumed is proportional to the COD of the sample.

Reagents

1. Standard potassium dichromate solution (0.25 N)
2. Concentrated sulfuric acid (H_2SO_4)
3. Silver sulfate (Ag_2SO_4) - as a catalyst
4. Mercuric sulfate ($HgSO_4$) - to suppress chloride interference
5. Ferrous ammonium sulfate (FAS) solution (0.1 N)
6. Ferroin indicator
7. Distilled water
8. Standard ferrous ammonium sulfate (FAS) solution

Apparatus

1. Reflux apparatus
2. Burette
3. Pipette
4. Conical flask
5. Volumetric flask
6. Heating mantle or water bath
7. Glass rod
8. Erlenmeyer flasks

Procedure

1. **Preparation of Reagents:**
 - **Potassium dichromate solution (0.25 N):** Dissolve an appropriate amount of $K_2Cr_2O_7$ in distilled water to make a 0.25 N solution.

- **Ferrous ammonium sulfate (FAS) solution (0.1 N):** Dissolve an appropriate amount of FAS in distilled water to make a 0.1 N solution.
 - **Ferriin indicator:** Typically prepared as a 0.025 M solution.
2. **Sample Preparation:**
- If necessary, filter the water sample to remove any suspended solids.
3. **Digestion:**
- Pipette 50 mL of the water sample into a reflux flask.
 - Add 1 g of mercuric sulfate (HgSO_4) to the flask to suppress chloride interference.
 - Add 5 mL of concentrated sulfuric acid (H_2SO_4) containing silver sulfate (Ag_2SO_4) to the flask as a catalyst.
 - Add 25 mL of 0.25 N potassium dichromate solution to the flask.
 - Mix the contents thoroughly.
 - Attach the reflux flask to the reflux apparatus and heat the mixture for 2 hours.
4. **Titration:**
- After the digestion, allow the mixture to cool to room temperature.
 - Transfer the contents of the reflux flask to an Erlenmeyer flask, rinsing the reflux flask with distilled water and adding the rinse to the Erlenmeyer flask.
 - Add 2-3 drops of ferriin indicator to the mixture.
 - Titrate the solution with 0.1 N ferrous ammonium sulfate (FAS) until the color changes from blue-green to reddish-brown (the endpoint).
5. **Calculation:**
- Note the volume of FAS used in the titration.
 - Calculate the COD using the formula:

$$\text{COD (mg/L)} = \frac{(a - b) \times N \times 8000}{\text{Volume of sample (mL)}}$$
 Where:
 - a = Volume of FAS used for the blank (mL)
 - b = Volume of FAS used for the sample (mL)
 - N = Normality of FAS solution

Results

Record the volumes of FAS used for both the blank and the water sample. Calculate the COD values and present the results in a tabular form:

Sample ID	Volume of FAS for Blank (mL)	Volume of FAS for Sample (mL)	COD (mg/L)
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Analyze the results to determine the COD levels of the different water samples. Compare the values to standard COD levels to assess the water

Estimation of Biochemical Oxygen Demand (BOD)

Aim : To estimate the Biochemical Oxygen Demand (BOD) of a water sample, which measures the amount of dissolved oxygen consumed by microorganisms during the oxidation of organic matter over a specific period.

Principle : Biochemical Oxygen Demand (BOD) is the amount of oxygen required by aerobic microorganisms to decompose organic matter in water over a specified period, usually 5 days at 20°C. BOD is an indicator of the organic pollution level in water. The test involves measuring the dissolved oxygen (DO) content of the sample before and after incubation.

Reagents

1. Manganous sulfate (MnSO_4) solution
2. Alkaline potassium iodide solution
3. Sulfuric acid (H_2SO_4)
4. Sodium thiosulfate solution (0.025 N)
5. Starch indicator solution
6. Phosphate buffer solution
7. Magnesium sulfate solution
8. Calcium chloride solution
9. Ferric chloride solution
10. Distilled water

Apparatus

1. BOD bottles (300 mL)
2. Incubator (maintained at 20°C)
3. Pipettes
4. Burette
5. Conical flask
6. Oxygen meter or titration setup for DO determination
7. Volumetric flasks
8. Thermometer

Procedure

1. **Sample Collection:**
 - Collect water samples in BOD bottles without trapping air bubbles to avoid oxygen contamination.
2. **Initial DO Determination (Day 0):**
 - Add 1 mL of manganous sulfate (MnSO_4) solution to the sample.
 - Add 1 mL of alkaline potassium iodide solution.

- Add 1 mL of sulfuric acid (H₂SO₄) to dissolve the precipitate formed.
 - Mix well and titrate with 0.025 N sodium thiosulfate solution until the yellow color fades.
 - Add a few drops of starch indicator solution and continue titrating until the blue color disappears.
 - Record the volume of sodium thiosulfate used (V1).
- 3. Incubation:**
- Prepare dilution water by adding phosphate buffer, magnesium sulfate, calcium chloride, and ferric chloride solutions to distilled water.
 - Fill the BOD bottles with the water sample, leaving no air space.
 - Incubate the bottles at 20°C for 5 days.
- 4. Final DO Determination (Day 5):**
- After 5 days, determine the DO in the incubated sample using the same method as for the initial DO determination.
 - Record the volume of sodium thiosulfate used (V2).
- 5. Calculation:**
- Calculate the BOD using the formula:

$$\text{BOD (mg/L)} = (V1 - V2) \times \frac{N \times 8000}{\text{Volume of sample (mL)}}$$
- Where:
- V1 = Volume of sodium thiosulfate used for initial DO determination (mL)
 - V2 = Volume of sodium thiosulfate used for final DO determination (mL)
 - N = Normality of sodium thiosulfate solution

Results

Record the initial and final DO values for each sample. Calculate the BOD values and present the results in a tabular form:

Sample ID	Initial DO (mg/L)	Final DO (mg/L)	BOD (mg/L)
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Analyze the results to determine the BOD levels of the different water samples. Compare the values to standard BOD levels to assess the water quality.

Determination of Suspended Solids in Industrial Effluents

Aim : To determine the concentration of suspended solids in industrial effluents.

Principle : Suspended solids (SS) are solid particles that are suspended in water and can be trapped by a filter. The concentration of suspended solids is an important parameter for assessing water quality. In this method, a known volume of water sample is filtered through a pre-weighed filter paper. The filter paper is then dried and weighed again. The increase in weight corresponds to the amount of suspended solids in the sample.

Reagents

1. Distilled water
2. Industrial effluent samples

Apparatus

1. Filter apparatus (e.g., vacuum filtration setup)
2. Pre-weighed filter papers (usually of known pore size, e.g., 0.45 μm)
3. Drying oven
4. Desiccator
5. Analytical balance
6. Beakers
7. Graduated cylinder

Procedure

1. **Preparation of Apparatus:**
 - Assemble the filter apparatus and ensure it is clean.
 - Dry the filter paper in the oven at 103-105°C for 1 hour to remove any moisture.
 - Cool the filter paper in a desiccator and then weigh it using an analytical balance. Record the initial weight (W1).
2. **Sample Filtration:**
 - Measure a known volume of the industrial effluent sample (e.g., 100 mL) using a graduated cylinder.
 - Pour the measured sample into the filtration apparatus and filter it through the pre-weighed filter paper under vacuum.
 - Rinse the graduated cylinder and the filter apparatus with distilled water to ensure all suspended solids are transferred to the filter paper.
3. **Drying and Weighing:**
 - Carefully remove the filter paper with the retained solids and place it in the drying oven.
 - Dry the filter paper at 103-105°C for at least 1 hour to remove any moisture.

- Cool the filter paper in a desiccator and weigh it again. Record the final weight (W₂).

4. Calculation:

- Calculate the concentration of suspended solids using the formula:
Suspended Solids (mg/L) = $\frac{(W_2 - W_1) \times 1000}{\text{Volume of sample (mL)}}$

$$\text{Suspended Solids (mg/L)} = \frac{(W_2 - W_1) \times 1000}{\text{Volume of sample (mL)}}$$

Where:

- W₁ = Initial weight of the filter paper (mg)
- W₂ = Final weight of the filter paper with dried solids (mg)
- Volume of sample (mL) is the volume of the industrial effluent filtered.

Results

Record the initial and final weights of the filter paper for each sample. Calculate the concentration of suspended solids and present the results in a tabular form:

Sample ID	Volume of Sample (mL)	Initial Weight (W ₁) (mg)	Final Weight (W ₂) (mg)	Suspended Solids (mg/L)

Analyze the results to determine the concentration of suspended solids in the different industrial effluent samples. Compare the values to standard permissible limits to assess the quality of the effluents.

Removal of Color of Industrial Effluents by Biological Methods

Aim : To remove the color of industrial effluents using biological methods such as the use of specific bacteria, fungi, or algae capable of degrading or adsorbing color-causing compounds.

Principle : Color removal from industrial effluents using biological methods relies on the metabolic activities of microorganisms that can degrade or adsorb the color-causing compounds. Microorganisms like bacteria, fungi, and algae can break down complex organic dyes into simpler, less harmful compounds through enzymatic actions. This method is environmentally friendly and cost-effective compared to chemical and physical methods.

Reagents

1. Industrial effluent samples
2. Nutrient media for microbial growth (e.g., nutrient broth, potato dextrose agar)
3. Specific microorganisms (e.g., bacteria like *Pseudomonas* sp., fungi like *Phanerochaete chrysosporium*, algae like *Chlorella* sp.)
4. Distilled water

Apparatus

1. Incubator or shaker
2. Erlenmeyer flasks
3. Sterile petri dishes
4. Autoclave
5. Spectrophotometer
6. pH meter
7. Sterile pipettes
8. Beakers
9. Inoculating loop

Procedure

1. **Preparation of Microbial Culture:**
 - Obtain pure cultures of the microorganisms to be used (e.g., bacteria, fungi, algae).
 - Prepare nutrient media according to standard protocols and sterilize by autoclaving.
 - Inoculate the media with the microorganisms and incubate under optimal growth conditions (e.g., 30°C for bacteria, 25°C for fungi) until a significant growth is observed.
2. **Preparation of Effluent Samples:**
 - Collect industrial effluent samples and filter to remove large particulates.

- Measure the initial color intensity of the effluent using a spectrophotometer at the appropriate wavelength (e.g., 465 nm for yellow dyes, 620 nm for blue dyes).

3. Treatment with Microorganisms:

- Inoculate the effluent samples with the prepared microbial cultures in Erlenmeyer flasks.
- Maintain controls with non-inoculated effluent samples.
- Incubate the flasks under optimal conditions for the microorganisms (e.g., shaking at 120 rpm for bacteria, static incubation for fungi) for a specific period (e.g., 7 days).

4. Monitoring and Measurement:

- Regularly monitor the pH and temperature of the effluent samples during the incubation period.
- After the incubation period, measure the color intensity of the treated and control effluent samples using a spectrophotometer.
- Calculate the percentage color removal using the formula:

$$\text{Color Removal (\%)} = \frac{(\text{Initial Absorbance} - \text{Final Absorbance})}{\text{Initial Absorbance}} \times 100$$

Initial Absorbance

Results

Record the initial and final absorbance values of the effluent samples for each microorganism treatment and calculate the percentage color removal. Present the results in a tabular form:

Sample ID	Microorganism Used	Initial Absorbance	Final Absorbance	Color Removal (%)

Analyze the results to determine the effectiveness of different microorganisms in removing color from the industrial effluent samples. Compare the values to assess which microorganism is the most efficient.

Reduction of Pollution Load in Effluents by Biological Methods

Aim: To reduce the pollution load in industrial effluents using biological methods such as bioremediation with specific bacteria, fungi, or algae capable of degrading or transforming pollutants.

Principle : Biological methods for pollution reduction involve the use of microorganisms to degrade or transform pollutants into less harmful forms. These microorganisms metabolize organic and inorganic pollutants through enzymatic reactions, leading to the detoxification of the effluents. The efficiency of pollutant removal depends on the type of microorganisms used, the nature of the pollutants, and the environmental conditions.

Reagents

1. Industrial effluent samples
2. Nutrient media for microbial growth (e.g., nutrient broth, potato dextrose agar)
3. Specific microorganisms (e.g., bacteria like *Pseudomonas* sp., fungi like *Phanerochaete chrysosporium*, algae like *Chlorella* sp.)
4. Distilled water
5. Buffer solutions for pH adjustment (if needed)
6. Analytical reagents for measuring pollutant concentrations (e.g., COD, BOD, heavy metals)

Apparatus

1. Incubator or shaker
2. Erlenmeyer flasks
3. Sterile petri dishes
4. Autoclave
5. Spectrophotometer
6. pH meter
7. Analytical balance
8. DO meter (for BOD measurement)
9. COD digestion apparatus
10. Atomic absorption spectrophotometer (for heavy metals)
11. Sterile pipettes
12. Beakers
13. Inoculating loop

Procedure

1. **Preparation of Microbial Culture:**
 - Obtain pure cultures of the microorganisms to be used (e.g., bacteria, fungi, algae)

- Prepare nutrient media according to standard protocols and sterilize by autoclaving.
 - Inoculate the media with the microorganisms and incubate under optimal growth conditions (e.g., 30°C for bacteria, 25°C for fungi) until a significant growth is observed.
- 2. Preparation of Effluent Samples:**
- Collect industrial effluent samples and filter to remove large particulates.
 - Measure initial pollution parameters such as COD, BOD, and concentrations of specific pollutants like heavy metals.
- 3. Treatment with Microorganisms:**
- Inoculate the effluent samples with the prepared microbial cultures in Erlenmeyer flasks.
 - Maintain controls with non-inoculated effluent samples.
 - Incubate the flasks under optimal conditions for the microorganisms (e.g., shaking at 120 rpm for bacteria, static incubation for fungi) for a specific period (e.g., 7 days).
- 4. Monitoring and Measurement:**
- Regularly monitor the pH and temperature of the effluent samples during the incubation period.
 - After the incubation period, measure the pollution parameters (COD, BOD, and concentrations of specific pollutants) in the treated and control effluent samples.
- 5. Calculation:**
- Calculate the percentage reduction in pollution load using the formula:

$$\text{Pollution Reduction (\%)} = \left(\frac{\text{Initial Value} - \text{Final Value}}{\text{Initial Value}} \right) \times 100$$

Results

Record the initial and final values of the pollution parameters for each microorganism treatment and calculate the percentage reduction in pollution load. Present the results in a tabular form:

Sample ID	M.O's Used	Initial COD (mg/L)	Final COD (mg/L)	Initial BOD (mg/L)	Final BOD (mg/L)	BOD Reduction (%)	Initial Heavy Metal Concentration (mg/L)	Final Heavy Metal Concentration (mg/L)	Heavy Metal Reduction (%)

Analyze the results to determine the effectiveness of different microorganisms in reducing the pollution load in the industrial effluent samples. Compare the values to assess which microorganism is the most efficient.

Polymerase Chain Reaction (PCR)

Aim : To amplify a specific segment of DNA using the Polymerase Chain Reaction (PCR) technique.

Principle : Polymerase Chain Reaction (PCR) is a molecular biology technique used to amplify specific DNA sequences. It involves repeated cycles of denaturation, annealing, and extension. During these cycles, a DNA polymerase enzyme synthesizes new DNA strands complementary to the target sequence, exponentially increasing the number of DNA copies.

Reagents

1. DNA template (sample containing the target DNA sequence)
2. Forward primer (a short single-stranded DNA complementary to the 5' end of the target sequence)
3. Reverse primer (a short single-stranded DNA complementary to the 3' end of the target sequence)
4. Deoxynucleotide triphosphates (dNTPs) mix (dATP, dCTP, dGTP, dTTP)
5. DNA polymerase (Taq polymerase or another thermostable polymerase)
6. PCR buffer (containing MgCl₂ and other necessary components)
7. Nuclease-free water

Apparatus

1. Thermal cycler (PCR machine)
2. Microcentrifuge tubes
3. Pipettes and tips
4. Gel electrophoresis apparatus (for analyzing PCR products)
5. Agarose gel
6. Ethidium bromide or another DNA stain
7. UV transilluminator (for visualizing stained DNA)

Procedure

1. Preparation of PCR Reaction Mix:

- Thaw all reagents on ice.
- Prepare the PCR reaction mix in a microcentrifuge tube. A typical reaction (total volume 25 µL) may include:
 - 1-5 µL DNA template
 - 0.5-1 µL forward primer (10 µM)
 - 0.5-1 µL reverse primer (10 µM)
 - 0.5 µL dNTPs mix (10 mM each)
 - 0.5-1 µL DNA polymerase (e.g., Taq polymerase)
 - 2.5 µL 10X PCR buffer

- Nuclease-free water to make up the final volume to 25 μ L
 - Mix gently by pipetting up and down.
- 2. **PCR Cycling Conditions:**
 - Place the reaction tube in the thermal cycler and set the cycling conditions. A typical PCR program consists of:
 1. Initial denaturation: 95°C for 2-5 minutes
 2. 30-35 cycles of:
 - Denaturation: 95°C for 30 seconds
 - Annealing: 50-65°C for 30 seconds (depending on the melting temperature of the primers)
 - Extension: 72°C for 1 minute (time depends on the length of the target sequence, typically 1 minute per 1 kb of DNA)
 3. Final extension: 72°C for 5-10 minutes
 4. Hold: 4°C (indefinitely, until samples are removed)

Analysis of PCR Products:

Prepare an agarose gel (1-2%) and set up the gel electrophoresis apparatus.

Mix a portion of the PCR product with a DNA loading dye.

Load the samples into the wells of the agarose gel along with a DNA ladder (molecular weight marker).

Run the gel at an appropriate voltage (e.g., 100-120 V) until the dye has migrated sufficiently.

Stain the gel with ethidium bromide or another DNA stain.

Visualize the DNA bands under a UV transilluminator.

Results

Record the presence and size of the amplified DNA bands. Compare the bands to the DNA ladder to estimate the size of the PCR products. Present the results in a tabular form:

Sample ID	Expected Size (bp)	Observed Size (bp)	Amplification Success (Yes/No)
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Analyze the results to determine the success of the PCR amplification. Confirm that the observed band size matches the expected size for the target DNA sequence.