DISSOLVED OXYGEN ANALYSIS AND BIOCHEMICAL OXYGEN DEMAND

1. The purpose of this experiment is to determine the BOD_7 value that is used as a criterion for the biodegradable pollutants present in water by measuring the dissolved oxygen concentration at day=0 and day=7.

2. Theoretical Background

Biochemical oxygen demand (BOD) is a measure of the amount of oxygen required by bacteria to oxidize waste aerobically to simple endproducts such as carbon dioxide, water and ammonia (1).

Microorganisms + Organic Matter + O_2 → More Microorganisms + CO_2 + H_2O + NH_3

BOD_5 determination is made by addition of a known and sufficient quantity of elemental oxygen-saturated dilution water such that at the end of the incubation time, a residual amount of at least 1 mg/L oxygen is left. BOD_5 parameter requires the presence of bacterial inoculum capable of degrading the organic matter present in the sample (acclimation).

Theoretically, an infinite time is necessary to complete biological oxidation but for practical purposes **a period of 5 days** is found satisfactory. **In this lab, we will have an incubation time of 7 days**. The rate of oxidation may be given as a first order reaction:

$$y = L\,[1-e^{-kt}]$$

where

y: BOD at any given time (mg/L)
L: Ultimate BOD (mg/L)
k: First order rate (decay) constant (d^{-1})
t: Time in days (d)

BOD relies on the measurement of dissolved oxygen (DO) in the sample at the beginning and the end of an incubation period and is given in mg O_2/L. Dissolved oxygen is an important parameter in water quality considerations (Salmon needs almost saturated conditions ~8.5 mg/L) and for the control of wastewater treatment plant efficiencies. In addition, the DO parameter is used in corrosion control. The DO concentration in a water body is a function of salinity and temperature and is inversely related to these parameters. The DO concentrations can be measured either by a DO probe or through the Winkler method with Azide modification. The reactions involved are:

$$2\,Mn^{2+} + 4\,OH^- + O_2 \rightarrow MnO_2\downarrow + 2\,H_2O$$

Brown precipitate

$$MnO_2\downarrow + 4H^+ + 2\,I^- \rightarrow I_2 + Mn^{2+} + 2\,H_2O$$

Brown solution

$$I_2 + 2\,S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2I^-$$

The Azide modification (addition of sodium azide) is used to eliminate the interference that may be caused by nitrite present in the sample.

$$6N_3^- + NO_2^- + 8\ H^+ \quad 10\ N_2 + 4\ H_2O$$

If there is no oxygen in the water, a white precipitate is formed, indicating the end of the experiment-without any quantitative result!

$$2\ Mn^{2+} + 4\ OH^- \quad Mn(OH)_2 \downarrow \text{ White precipitate}$$

3. Experimental Set-up

Titration stand (+ burettes), erlenmeyer flasks, funnels, BOD bottles, pipettes (regular and wide bore), graduated cylinder, incubator (set @20^{o}C)

4. Reagents

a. Distilled water
b. Manganese sulfate

Dissolve either 480g $MnSO_4.4H_2O$ or 400g $MnSO_4.2H_2O$ or 364 $MnSO_4.H_2O$ in water and dilute to 1000 mL. This solution should stay colorless upon addition of acidified potassium iodide.

c. Alkaline-iodide-azide reagent

Dissolve 500 g NaOH and 150 g KI in distilled water. Add 10 g sodium azide (NaN_3) dissolved in 40mL distilled water. Dilute to 1000 mL. This solution should not produce color upon dilution or acidification.

d. Concentrated sulphuric acid

1 mL of concentrated sulfuric acid is equal to 3 mL of Alkaline-iodide-azide reagent

e. Starch solution

Dissolve 2 g of starch in distilled water, add 0.2 salicylic acid (preservative) and dilute to 100 mL.

f. Standard Sodium thiosulfate solution (0.025 N)

Dissolve 6.205 g Na2S2O3.5H2O in freshly boiled and cooled distilled water and dilute to 1000 mL. Standardize against the standard potassium dichromate solution.

g. Standard Potassium diiodide solution (0.0021 M)

Dissolve 812.4 mg $KH(IO_3)_2$ in distilled water and dilute to 1000 mL.

Standardization of sodium thiosulfate solution:
Dissolve 2 g KI in 100 mL distilled water and add 10 mL (1+3) H_2SO_4 to this solution. Add 20 mL standard dichromate solution and dilute to 200 mL. Titrate liberated iodine with the thiosulfate titrant; add 2mL starch solution towards the end of titration when a pale straw color is reached. 20 mL of thiosulfate should be used for titration.

h. Potassium fluoride solution

Dissolve 40 g $KF.2H_2O$ in distilled water and dilute to 100 mL.

i. Phosphate buffer solution (pH:7.2)

Dissolve 0.85 g KH_2SO_4, 2.175 g K_2HPO_4, 3.34 g Na_2HPO_4 and 1.17 g NH_4Cl in distilled water and dilute to 250 mL. The final pH should be 7.2 without further adjustment.

j. Magnesium sulfate solution

Dissolve 2.25 g $MgSO_4.7H_2O$ in distilled water and dilute to 100 mL.

k. Calcium chloride solution

Dissolve 2.75 g anhydrous $CaCl_2$ in distilled water and dilute to 100 mL.

l. Ferric chloride solution

Dissolve 0.25 g $FeCl_3.6H_2O$ in distilled water and dilute to 100 mL.

5. Experimental Procedure

In general, the BOD test requires that there is no toxicity during the experiments and all the required nutrients are supplied to the bacteria for growth to make sure that the restricting nutrient is organic carbon. Dilution is required to assure that not all the dissolved oxygen will be depleted during the incubation period.

A. Preparation of BOD bottles

Using the table and your judgment, determine how much dilution will be needed to make sure that there will be a minimum O_2 concentration of 1 mg/L O_2 in the BOD bottles at the end of the incubation period and at least 2 mg/L O_2 will be used during

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the incubation. Prepare three dilutions for each sample and triplicates for each dilution. One of the bottles will be used for the day=0 DO measurement, whereas the DO in the other two bottles will be measured at the end of the incubation period. Pour the calculated amounts of sample and dilution water into a graduated cylinder

and add 1mL of each of the following 4 solutions to 1 L of dilution water: $MgSO_4$ solution, $CaCl_2$, $FeCl_3$, and phosphate buffer solution. Add dilution water to bring the total volume to 1L. If needed add approximately 1-2 mL of bacterial inocula (this addition should not require a correction of 0.6 mg/L) before adding the dilution water. If the pH of the sample is not between 6.5-8.5, a pH correction is needed using 1N NaOH or 1N H_2SO_4 to bring the pH of the sample to 7.0.

Fill three BOD bottles with dilution water and add appropriate volumes of $MgSO_4$ solution, $CaCl_2$, $FeCl_3$, and phosphate buffer solution. The blank is used to check that the dilution water has negligible BOD (less than 0.2 mg/L O_2 use in 5 days).

If seed (bacterial inocula) is to be used, then prepare two sets of seed control samples with 2% and 5% seed in dilution water.

Stopper the BOD bottles, being sure to exclude all air bubbles. All bottles should have liquid in the well around the stopper - if not add some dilution water to the well. Place caps over all bottles. These caps are designed to prevent the evaporation of the liquid around the stopper, thereby maintaining a water seal for each bottle. Place two bottles for each dilution set + one of the blanks+ one of the seed controls in the $20^{O}C$ incubator for seven **(normally five!)** days. One bottle of each dilution set, one of the blanks and one of the seed controls will be used to measure the D.O at day=0.

Expected BOD (mg/L O_2)	**Dilution factor (%)**
20 000-70 000	0.01
10 000-35 000	0.02
4 000-14 000	0.05
2 000-7 000	0.10
1 000-3 500	0.20
400-1 400	0.50
200-700	1.00
100-350	2.00
40-140	5.00
20-70	10.00
10-35	20.00
4-14	50.00
0-7	100.00

B. Measurement of DO concentrations

Add 1 mL of $MnSO_4$ solution and 1 mL alkaline azide iodide solution to the sample just underneath the surface. Stopper carefully and mix by inverting the bottle at least

15 times. When the precipitate settles, leaving a clear supernatant above the MnO_2 flocs, add 1 mL of concentrated sulfuric acid. Stopper the bottle again and shake until all the precipitates dissolve leaving a transparent solution. Take 200 mL to a clean erlenmeyer flask and titrate with 0.025 N standard sodium thiosulfate to a pale straw color. Add 1-2 mL of starch solution; the solution will become blue. Continue the titration to the disappearance of the blue color and note the volume of the titrant used.

6. Expression of Results

Report your results in terms of mg/L O_2

Dissolved oxygen concentration:

Dissolved oxygen (mg/L O_2) = A × N × 8× 1000 / mL sample

Biochemical oxygen demand:

BOD_5 (mg/L O_2) = $(DO_1 - DO_2)$/ P (without any wastewater addition)

BOD_5 (mg/L O_2) = $(DO_1 – DO_2) - (B_1 - B_2) \times f$ / P

DO_1 : Dissolved oxygen concentration of the sample at day:0, mg/L
DO_2 : Dissolved oxygen concentration of the sample at day:7, mg/L
B_1 : Dissolved oxygen concentration of the seed control at day:0, mg/L
B_2 : Dissolved oxygen concentration of the seed control at day:7, mg/L
P : Percent fraction of sample
f : Ratio of the amount of seed in the sample to the amount of seed in the seed control (% Seed in DO_1 / % Seed in B_1)