**DISSOLVED OXYGEN**

The **Winkler test** is used to determine the concentration of dissolved [oxygen](http://en.wikipedia.org/wiki/Oxygen%22%20%5Co%20%22Oxygen) in water samples. The Winkler Method uses titration to determine dissolved oxygen in the water sample. A sample bottle is filled completely with water (no air is left to skew the results). The dissolved oxygen in the sample is then "fixed" by adding a series of reagents that form an acid compound that is then titrated with a neutralizing compound that results in a color change. The point of color change is called the "endpoint," which coincides with the dissolved oxygen concentration in the sample.

Reactions;

If the sample doesn’t contain oxygen, when MnSO4 and alkali iodide (NaOH + KI) was added, the white Mn(OH)2 flocs will ocur.

*Mn*+*2* + *2OH* →*Mn(OH )2* ↓ (*White precipitate*)

If the sample contain oxygen, when MnSO4 and alkali iodide (NaOH + KI) was added, the brown MnO2 flocs will ocur.

*Mn*+*2* + *2OH +1/2O2* →*MnO)2* ↓ +H2O (*brown precipitate*)

***Materials and Equipments***

Burette

Pipette

Incubation bottles

Erlenmayer for titration

Measuring cylinder

***Reagents***

Manganese sulfate

Alkali-iodide-azide

Concentrated sulfuric acid

Starch solution

Sodium thiosulfate

***Procedure***

1. Carefully fill a 300-mL glass Biological Oxygen Demand (BOD) bottle brim-full with sample water.
2. Add 1mL of manganese sulfate to the bottle by inserting the pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via the pipette.
3. Add 1 mL of alkali-iodide-azide reagent in the same manner.
4. Check the bottles for air bubbles; there musn’t be air bubbles. **If oxygen is present, a brownish-orange cloud of precipitate or floc will appear. When this floc has settle to the bottom, mix the sample by turning it upside down several times and let it settle again.**
5. Add 1 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully mix the sample several times to dissolve the floc.
6. Take 200 ml of the sample to a erlanmayer.
7. Start titration the sample with sodium thiosulfate to a light yellow color.
8. Add 2 drops of starch solution so a blue color forms.
9. Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue color..
10. The concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used. Each mL of sodium thiosulfate added in steps 7 and 9 equals 1 mg/L dissolved oxygen.

1 ml 0,025N Na2S2O3(sodium thiosulfate) = 1 mg/L Dissolved Oxygen

**BIOCHEMICAL OXYGEN DEMAND (BOD)**

In the presence of free oxygen, aerobic bacteria use the organic matter found in wastewater as “food”. The BOD test is an estimate of the “food” available in the sample. The more “food” present in the wastewater, the more Dissolved Oxygen (DO) will be required. The BOD test measures the strength of the wastewater by measuring the amount of oxygen used by the bacteria as they stabilize the organic matter under controlled conditions of time and temperature.

The DO content is determined and recorded and the bottle is incubated in the dark for five days at 20°C. At the end of five days, the final DO content is determined and the difference between the final DO reading and the initial DO reading is calculated. The decrease in DO is corrected for sample dilution, and represents the biochemical oxygen demand of the sample

**Why 5-Day BOD Test? The experimental results show that after 5 days measured BOD is approximately equal to 70% and 80% of total BOD so we accept the 5-day BOD value in the experiment.**

***Materials and Equipments***

Burette

Pipette

Incubation bottles

Erlenmayer for titration

Measuring cylinder

***Reagents***

Dilution water

Manganese sulfate

Alkali-iodide-azide

Concentrated sulfuric acid

Starch solution

Sodium thiosulfate

***Procedure***

1. Chose the dilution rate from table according to approximately BOD value of the sample. For example; approximately BOD value of the sample is 7500 mg/L. It shows the 0,05. From the sample for 1L, (0,05/100)x1000=0,5 ml must be taken. For dilution, under and above of 0,05 from the table must be prepared for correction.
2. Take the sample to measuring cylinder which found in Step 1
3. Add 1 ml seed and fill the measuring cylinder to 1L with dilution water
4. Fill the incubation bottles brim-full with this sample.
5. Check the bottles for air bubbles, there musn’t be air bubbles.
6. Close the stopper of bottles and add water to prevent the oxygen transfer to sample from air.
7. Take 3 of that samples, and measure the dissolved oxygen concentration by winkler method in maximum 30 minutes immediately to know initial dissolved oxygen concentration.

***After 5-days***

1. Take the bottles from incubator.
2. Add 1mL of manganese sulfate to the bottle by inserting the pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via the pipette.
3. Add 1 mL of alkali-iodide-azide reagent in the same manner.
4. Check the bottles for air bubbles; there musn’t be air bubbles. **If oxygen is present, a brownish-orange cloud of precipitate or floc will appear. When this floc has settle to the bottom, mix the sample by turning it upside down several times and let it settle again.**
5. Add 1 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully mix the sample several times to dissolve the floc.
6. Take 200 ml of the sample to a erlanmayer.
7. Start titration the sample with sodium thiosulfate to a light yellow color.
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10. The concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used. Each mL of sodium thiosulfate added in steps 7 and 9 equals 1 mg/L dissolved oxygen.

