

Determination of Dissolved Oxygen Concentration

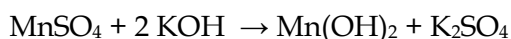
The measurement of dissolved oxygen is one of the most important of all determinations done in limnology. The amount of dissolved oxygen in the water can be an important factor controlling the distribution of aquatic organisms, and so knowing the amount of oxygen in the water can be useful for understanding or managing the spatial distribution of species in a lake. For example, dissolved oxygen concentrations must be about 8-15 mg/L in order to maintain adequate game fish populations. Determining the concentration of dissolved oxygen in a water sample is also used to estimate primary productivity.

The amount of oxygen that diffuses into water is in equilibrium with concentrations in the air. Air is about 20% oxygen by volume. The amount that may be dissolved in the water is dependent on the water temperature and the atmospheric pressure. Oxygen content of water is limited to about 11.3 mg/L at 10 °C, 9.2 mg/L at 20 °C, and 7.6 mg/L at 30 °C. Within the water column, oxygen concentration is increased by photosynthesis of plants, while concentrations are diminished by respiration and the demand for oxygen by abiotic chemical reactions.

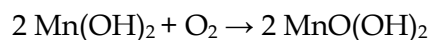
The two most frequently used methods of determining dissolved oxygen are the use of an oxygen electrode and the Winkler method. Using an electrode is more convenient, but not always very reliable because readings are influenced by factors such as pressure, temperature, and turbulence, and the electrodes are not capable of measuring very low concentrations. The Winkler method is more complicated and can be difficult to perform in the field, but it is much more accurate. We will use a field-friendly version of the Winkler method to measure changes in dissolved oxygen resulting from photosynthetic activity.

The Winkler Titration

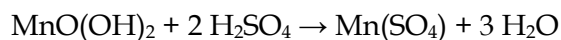
The Winkler titration was developed in the last century by L.W. Winkler of Budapest, Hungary. In this clever method, manganous sulfate reacts with a potassium hydroxide-potassium iodide mixture to produce a white flocculent precipitate of manganous hydroxide (bivalent manganese):



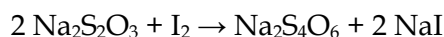
The precipitate reacts with dissolved oxygen to form tetravalent manganese:



This product, manganic basic oxide, is a brownish precipitate. A white precipitate indicates that no oxygen is present. Upon acidification with sulfuric acid (or sulfamic acid, H_3NSO_3) the precipitate dissolves, forming manganic sulfate:



A reaction of manganic sulfate and potassium iodide yields free iodine (I_2), which is a brownish color. The number of moles of iodine released is equivalent to the number of moles of dissolved oxygen originally present. Iodine also reacts with starch to produce a blue-black color which is used to diagnose the presence of iodine. The iodine may be titrated with sodium thiosulfate to yield sodium iodide, which no longer gives the brown color:



Thus, by measuring the amount of thiosulfate needed to remove all of the brown color, one determines the amount of I_2 in the sample, and indirectly the amount of dissolved O_2 originally present.

The first steps of the reaction – adding manganous sulfate, lithium hydroxide, potassium iodide, and sulfamic acid – must be done immediately after the sample is collected, before phytoplankton, bacteria, and other organisms can change the oxygen content through photosynthesis and respiration. Once the sample is “fixed” (all of the oxygen converted to iodine), the sample can be set aside for many hours before the titration with sodium thiosulfate is performed.

Methods

1. **Fill the BOD bottle** from the Hach kit (a small round bottle with a glass stopper) with the water to be tested. The sample should be introduced to the bottle **with as little contact with the air as possible** (why??). Normally, a siphon or delivery tube is used to fill the bottle once and then the delivery tube is carefully withdrawn. In lab, you will perform the tricky procedure of immersing the BOD bottle to elbow depth, filling it completely, and carefully stoppering it without introducing any air bubbles (or tipping the boat!). If bubbles are trapped in the bottle, discard the sample and start again.

2. Use the clippers in the kit to open one Dissolved Oxygen 1 Reagent Powder Pillow (manganous sulfate) and one Dissolved Oxygen 2 Reagent Powder Pillow (alkaline-iodide azide). **Add the contents of each pillow to the bottle**. Stopper the bottle carefully to exclude air bubbles. Grip the bottle and stopper firmly. Hold the stopper and **shake vigorously** to mix.

A reddish-brown flocculant (floc – fluffy particles) precipitate will form. If oxygen is present in the sample, the precipitate will be brownish-orange. A small amount of powdered reagent may remain stuck to the bottom of the bottle; this will not affect test results.

3. **Allow the sample to stand** until the floc has settled half-way in the bottle, leaving the upper half of the sample clear. **Shake the bottle again**. Let it stand until the upper half of the bottle is clear, about 4 or 5 minutes. Allowing the floc to settle a couple of times ensures that all of the chemicals are mixed and all of the dissolved oxygen has reacted.

4. Use the clippers to open one Dissolved Oxygen 3 Reagent Powder Pillow (sulfamic acid). Remove the stopper from the bottle and **add the contents**. Carefully restopper the bottle and **shake to mix**. The floc will dissolve. The solution will be yellow if there is oxygen present.

5. **Fill the plastic measuring tube** (5.83 ml) level full of the sample from step 4. Pour the sample into the square mixing bottle.

6. **Add sodium thiosulfate standard solution to the mixing bottle drop by drop**, swirling the bottle after each drop is added. Hold the dropper vertically above the bottle and **count each drop** added. Continue to add drops until the sample changes from yellow to colorless.

7. **Each drop added** to bring about the color change is equal to **1 mg/L** of dissolved oxygen.

8. If the results of step 7 indicate very low dissolved oxygen concentrations (< 3 mg/L), **a more sensitive test** should be performed. Use the prepared sample from step 4 above (the yellowish liquid left in the BOD bottle). **Pour off the contents until the level just reaches the mark on the bottle** (30 ml).

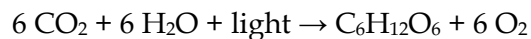
9. **Add sodium thiosulfate standard solution to the BOD bottle drop by drop**, swirling the bottle after each drop is added. Hold the dropper vertically above the bottle and **count each drop** added. Continue to add drops until the sample changes from yellow to colorless.

10. **Each drop added** to bring about the color change in this low range test is equal to **0.2 mg/L** of dissolved oxygen.

Algae: Primary Productivity

Primary productivity of a community, or the rate at which biomass is produced per unit area, is a way of estimating the biological activity of an ecosystem. Measures of productivity can be more useful than species composition or diversity when comparing very different ecosystems, since the amount of energy available places limits on the biological activity of any area. Primary productivity is measured in either units of energy (joules/m²/day) or dry organic matter (kg/ha/year).

Primary production by phytoplankton can be measured in one of two ways. Both ways are based on the stoichiometry of the photosynthesis reaction:



Determining the rate of consumption of CO₂ or the rate of production of O₂ thus can be used to determine the quantity of carbon fixed by photosynthesis.

For both methods (measuring CO₂ consumed or O₂ produced), the usual procedure is to enclose natural water samples in some kind of closed container and then measure changes in the concentration of O₂ or CO₂. The containers are often incubated *in situ* (placed back in the environment from which they came) or incubated in the laboratory under defined light and temperature conditions that simulate natural conditions.

There are other methods of estimating primary productivity. For example, it is possible to measure the changes in oxygen or carbon dioxide concentrations before, during, and after stratification (e.g. Areal Hypolimnetic Oxygen Deficit method, or AHOD). These methods are complicated by the fact that these gases diffuse in and out of the water column from the air. Other methods of estimating primary productivity are even more indirect and are based on long-term changes in system characteristics, such as biomass or nutrient content.

We will use the “light and dark bottle method” to determine rates of photosynthesis. We will incubate two kinds of bottles in the pond: light bottles and dark bottles. In the dark bottles, which allow no light in, there should be no photosynthesis and so the algae are only respiring. (We assume that they respire at the same rate whether they are photosynthesizing or not.) Thus the change in oxygen concentration in the dark bottle is the amount of oxygen consumed by respiration. In the light bottles, the algae will be photosynthesizing *and* respiring, so we will measure the **net** oxygen produced (total photosynthesis - respiration). The total oxygen produced, the **gross** photosynthesis, can then be estimated by summing the net change in oxygen concentration in the light bottle and the oxygen used in the dark bottle.

In other words, the total amount of energy produced through photosynthesis is the Gross Primary Productivity. The amount of energy left over after respiration is Net Primary Productivity (i.e. NPP = GPP - Respiration). Net Primary Productivity represents the rate of production of new biomass that is available to higher trophic levels.

Please see pages 212-217 in Dodson for more information about primary productivity and pages 32-33 and 44-45 for more about O₂ concentrations.

The containers we use are standard 300 ml glass BOD (biochemical oxygen demand) bottles with tapered ground glass stoppers. BOD bottles are so called because they were originally developed to be used for testing the oxygen requirements of waste water. The bottles will be incubated in the pond at a pre-selected depth (0.25 m).

Five BOD bottles will be filled. Two bottles will be titrated immediately to determine initial oxygen concentrations. Two more will be left uncovered and incubated in the pond, and the last bottle will be covered

Methods

1. Measure the intensity of light in the pond at the position where each bottle will be placed.
2. Fill five BOD bottles by immersing them to ~0.25 m below the surface (do not use surface water). The bottles should be filled carefully, and the stopper should be replaced without introducing any air into the bottles. (Technically, the best method to fill the BOD bottles is to siphon the water into the bottle, but we don't have time for this procedure.)

Two bottles are for the measurement of initial oxygen concentrations, and will be titrated immediately to determine initial oxygen concentrations. Two of these bottles will be replicates for light bottles, and will be incubated in the pond for about an hour. The last bottle will be covered in black tape or aluminum foil to eliminate any light, and also incubated in the pond. *Make sure you understand the purpose of all three bottle types!*

3. After filling the bottles, suspend them in the pond. Note the time and temperature. If the samples contain highly concentrated algae, watch the samples closely. After one hour, they may produce sufficient oxygen to exceed the solubility in the water and bubbles may form. This excess oxygen will not be measured and will spoil results because the amount of photosynthesis will be underestimated.
4. While the samples are incubating, measure the oxygen content of the initial samples. (See the accompanying handout about oxygen determinations.)
5. After the appropriate incubation period, remove the BOD bottles and determine the oxygen concentrations in the light and dark bottles using the Winkler titration.
6. Optional: While the samples are incubating determine the chlorophyll-a concentration of the sample. (See last week's handout about pigment analyses).

Calculations

The amount of **respiration** per unit volume per unit time is given by:

$$\text{respiration} = (\text{O}_2 \text{ initial bottle} - \text{O}_2 \text{ dark bottle}) / \text{hours incubated.}$$

The **net photosynthesis** per unit volume per unit time is given by:

$$\text{net photosynthesis} = (\text{O}_2 \text{ light bottle} - \text{O}_2 \text{ initial bottle}) / \text{hours incubated.}$$

Gross photosynthesis (net photosynthesis - respiration) is given by:

$$\begin{aligned} \text{gross photosynthesis} &= \text{net photosynthesis} + \text{respiration} \\ &= [(\text{O}_2 \text{ light} - \text{O}_2 \text{ initial}) + (\text{O}_2 \text{ initial} - \text{O}_2 \text{ dark})] / \text{hours} \end{aligned}$$

The terms for initial concentration cancel out so that you merely subtract the dark bottle concentration from the light bottle concentration.

1. Estimate the gross and net photosynthesis for Long Pond.
2. Estimated photosynthetic efficiency relates primary production rate to the amount of algae doing the producing. This is given by:

$$\text{net photosynthesis (mg O}_2\text{/L)} / \text{chl a (}\mu\text{g/L)} = \text{efficiency.}$$

Calculate the photosynthetic efficiency of the algae in Long Pond using your values of chlorophyll a content from last week. What does this measurement mean?

3. Another efficiency estimate relates net photosynthesis to both algal biomass and the amount of light available:

$$(\text{net photosynthesis}) \cdot (\text{chl a})^{-1} \cdot (\text{light intensity at bottles})^{-1} = \text{efficiency.}$$

How does this measure differ from the efficiency in #5 above?