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Water: Monitoring & Assessment

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5.2 Dissolved Oxygen and Biochemical Oxygen Demand

What is dissolved oxygen and why is it important?

The stream system both produces and consumes oxygen. It gains oxygen from the atmosphere and from plants as a result of photosynthesis. Running water, because of its churning, dissolves more oxygen than still water, such as that in a reservoir behind a dam. Respiration by aquatic animals, decomposition, and various chemical reactions consume oxygen.

Wastewater from sewage treatment plants often contains organic materials that are decomposed by microorganisms, which use oxygen in the process. (The amount of oxygen consumed by these organisms in breaking down the waste is known as the biochemical oxygen demand or BOD. A discussion of BOD and how to monitor it is included at the end of this section.) Other sources of oxygen-consuming waste include stormwater runoff from farmland or urban streets, feedlots, and failing septic systems.

Oxygen is measured in its dissolved form as dissolved oxygen (DO). If more oxygen is consumed than is produced, dissolved oxygen levels decline and some sensitive animals may move away, weaken, or die.

DO levels fluctuate seasonally and over a 24-hour period. They vary with water temperature and altitude. Cold water holds more oxygen than warm water (Table 5.3) and water holds less oxygen at higher altitudes. Thermal discharges, such as water used to cool machinery in a manufacturing plant or a power plant, raise the temperature of water and lower its oxygen content. Aquatic animals are most vulnerable to lowered DO levels in the early morning on hot summer days when stream flows are low, water temperatures are high, and aquatic plants have not been producing oxygen since sunset.

Sampling and Equipment Considerations

In contrast to lakes, where DO levels are most likely to vary vertically in the water column, the DO in rivers and streams changes more horizontally along the course of the waterway. This is especially true in smaller, shallower streams. In larger, deeper rivers, some vertical stratification of dissolved oxygen might occur. The DO levels in and below riffle areas, waterfalls, or dam spillways are typically higher than those in pools and slower-moving stretches. If you wanted to measure the effect of a dam, it would be important to sample for DO behind the dam, immediately below the spillway, and upstream of the dam. Since DO levels are critical to fish, a good place to sample is in the pools that fish tend to favor or in the spawning areas they use.

An hourly time profile of DO levels at a sampling site is a valuable set of data because it shows the change in DO levels from the low point just before sunrise to the high point sometime in the midday. However, this might not be practical for a volunteer monitoring program. It is important to note the time of your DO sampling to help judge when in the daily cycle the data were collected.

Table 5.3

Maximum dissolved oxygen concentrates vary with temperature

Temperature (°C)	DO (mg/l)	Temperature (°C)	DO (mg/l)
0	14.60	23	8.56
1	14.19	24	8.40
2	13.81	25	8.24
3	13.44	26	8.09
4	13.09	27	7.95
5	12.75	28	7.81
6	12.43	29	7.67
7	12.12	30	7.54
8	11.83	31	7.41
9	11.55	32	7.28
10	11.27	33	7.16
11	11.01	34	7.16

DO is measured either in milligrams per liter (mg/L) or "percent saturation." Milligrams per liter is the amount of oxygen in a liter of water. Percent saturation is the amount of oxygen in a liter of water relative to the total amount of oxygen that the water can hold at that temperature.

DO samples are collected using a special BOD bottle: a glass bottle with a "turtleneck" and a ground glass stopper. You can fill the bottle directly in the stream if the stream is wadable or boatable, or you can use a sampler that is dropped from a bridge or boat into water deep enough to submerse the sampler. Samplers can be made or purchased. Dissolved oxygen is measured primarily either by using some variation of the Winkler method or by using a meter and probe.

12	10.76	35	6.93
13	10.52	36	6.82
14	10.29	37	6.71
15	10.07	38	6.61
16	9.85	39	6.51
17	9.65	40	6.41
18	9.45	41	6.41
19	9.26	42	6.22
20	9.07	43	6.13
21	8.90	44	6.04
22	8.72	45	5.95

Winkler Method

The Winkler method involves filling a sample bottle completely with water (no air is left to bias the test). The dissolved oxygen is then "fixed" using a series of reagents that form an acid compound that is titrated. Titration involves the drop-by-drop addition of a reagent that neutralizes the acid compound and causes a change in the color of the solution. The point at which the color changes is the "endpoint" and is equivalent to the amount of oxygen dissolved in the sample. The sample is usually fixed and titrated in the field at the sample site. It is possible, however, to prepare the sample in the field and deliver it to a lab for titration.

Dissolved oxygen field kits using the Winkler method are relatively inexpensive, especially compared to a meter and probe. Field kits run between $35 and $200, and each kit comes with enough reagents to run 50 to 100 DO tests. Replacement reagents are inexpensive, and you can buy them already measured out for each test in plastic pillows.

You can also buy the reagents in larger quantities, in bottles, and measure them out with a volumetric scoop. The advantage of the pillows is that they have a longer shelf life and are much less prone to contamination or spillage. The advantage of buying larger quantities in bottles is that the cost per test is considerably less.

The major factor in the expense of the kits is the method of titration they use eyedropper, syringe-type titrator, or digital titrator. Eyedropper and syringe-type titration is less precise than digital titration because a larger drop of titrant is allowed to pass through the dropper opening and, on a micro-scale, the drop size (and thus the volume of titrant) can vary from drop to drop. A digital titrator or a buret (which is a long glass tube with a tapered tip like a pipet) permits much more precision and uniformity in the amount of titrant that is allowed to pass.

If your program requires a high degree of accuracy and precision in DO results, use a digital titrator. A kit that uses an eye dropper-type or syringe- type titrator is suitable for most other purposes. The lower cost of this type of DO field kit might be attractive if you are relying on several teams of volunteers to sample multiple sites at the same time.

Meter and Probe

A dissolved oxygen meter is an electronic device that converts signals from a probe that is placed in the water into units of DO in milligrams per liter. Most meters and probes also measure temperature. The probe is filled with a salt solution and has a selectively permeable membrane that allows DO to pass from the stream water into the salt solution. The DO that has diffused into the salt solution changes the electric potential of the salt solution and this change is sent by electric cable to the meter, which converts the signal to milligrams per liter on a scale that the volunteer can read.

DO meters are expensive compared to field kits that use the titration method. Meter/probe combinations run between $500 and $1,200, including a long cable to connect

the probe to the meter. The advantage of a meter/probe is that you can measure DO and temperature quickly at any point in the stream that you can reach with the probe. You can also measure the DO levels at a certain point on a continuous basis. The results are read directly as milligrams per liter, unlike the titration methods, in which the final titration result might have to be converted by an equation to milligrams per liter.

However, DO meters are more fragile than field kits, and repairs to a damaged meter can be costly. The meter/probe must be carefully maintained, and it must be calibrated before each sample run and, if you are doing many tests, in between samplings. Because of the expense, a volunteer program might have only one meter/probe. This means that only one team of samplers can sample DO and they will have to do all the sites. With field kits, on the other hand, several teams can sample simultaneously.

Laboratory Testing of Dissolved Oxygen

If you use a meter and probe, you must do the testing in the field; dissolved oxygen levels in a sample bottle change quickly due to the decomposition of organic material by microorganisms or the production of oxygen by algae and other plants in the sample. This will lower your DO reading. If you are using a variation of the Winkler method, it is possible to "fix" the sample in the field and then deliver it to a lab for titration. This might be preferable if you are sampling under adverse conditions or if you want to reduce the time spent collecting samples. It is also a little easier to titrate samples in the lab, and more quality control is possible because the same person can do all the titrations.

How to collect and analyze samples

The procedures for collecting and analyzing samples for dissolved oxygen consist of the following tasks:

TASK 1 Prepare before leaving for the sampling site

Refer to section 2.3 - Safety Considerations for details on confirming sampling date and time, safety considerations, checking supplies, and checking weather and directions. In addition to the standard sampling equipment and apparel, when sampling for dissolved oxygen, include the following equipment:

If Using the Winkler Method

- Labels for sample bottles
- Field kit and instructions for DO testing
- Enough reagents for the number of sites to be tested
- Kemmerer, Van Dorn, or home-made sampler to collect deep-water samples
- A numbered glass BOD bottle with a glass stopper (1 for each site)
- Data sheet for dissolved oxygen to record results

If Using a Meter and Probe

- DO meter and probe (electrode) (NOTE: Confirm that the meter has been calibrated according to the manufacturer's instructions.)
- Operating manual for the meter and probe
- Extra membranes and electrolyte solution for the probe
- Extra batteries for the meter
- Extension pole
- Data sheet for dissolved oxygen to record results

TASK 2 Confirm that you are at the proper location

The directions for sampling should provide specific information about the exact point in the stream from which you are to sample; e.g., "approximately 6 feet out from the large boulder downstream from the west side of the bridge." If you are not sure you are in the exact spot, record a detailed description of where you took the sample so that it can be compared to the actual site later.

TASK 3 Collect samples and fill out the field data sheet

Winkler Method

Use a BOD bottle to collect the water sample. The most common sizes are 300 milliliters (mL) and 60 mL. Be sure that you are using the correct volume for the titration method that will be used to determine the amount of DO. There is usually a white label area on the bottle, and this may already be numbered. If so, be sure to record that number on the field data sheet. If your bottle is not already numbered, place a label on the bottle (not on the cap because a cap can be inadvertently placed on a different bottle) and use a waterproof marker to write in the site number.

If you are collecting duplicate samples, label the duplicate bottle with the correct code, which should be determined prior to sampling by the lab supplying the bottles. Use the following procedure for collecting a sample for titration by the Winkler method:

1. Remember that the water sample must be collected in such a way that you can cap the bottle while it is still submerged. That means that you must be able to reach into the water with both arms and the water must be deeper than the sample bottle.
2. Carefully wade into the stream. Stand so that you are facing one of the banks.
3. Collect the sample so that you are not standing upstream of the bottle. Remove the cap of the BOD bottle. Slowly lower the bottle into the water, pointing it downstream, until the lower lip of the opening is just submerged. Allow the water to fill the bottle very gradually, avoiding any turbulence (which would add oxygen to the sample). When the water level in the bottle has stabilized (it won't be full because the bottle is tilted), slowly turn the bottle upright and fill it completely. Keep the bottle under water and allow it to overflow for 2 or 3 minutes to ensure that no air bubbles are trapped.
4. Cap the bottle while it is still submerged. Lift it out of the water and look around the "collar" of the bottle just below the bottom of the stopper. If you see an air bubble, pour out the sample and try again.
5. "Fix" the sample immediately following the directions in your kit:
   - Remove the stopper and add the fixing reagents to the sample.
   - Immediately insert the stopper so air is not trapped in the bottle and invert several times to mix. This solution is caustic. Rinse your hands if you get any solution on them. An orange-brown flocculent precipitate will form if oxygen is present.
   - Wait a few minutes until the floc in the solution has settled. Again invert the bottle several times and wait until the floc has settled. This ensures complete reaction of the sample and reagents. The sample is now fixed, and atmospheric oxygen can no longer affect it. If you are taking the sample to the lab for titration, no further action is necessary. You can store the sample in a cooler for up to 8 hours before titrating it in a lab. If you are titrating the sample in the field, see *Task 4: Analyze the Samples.*

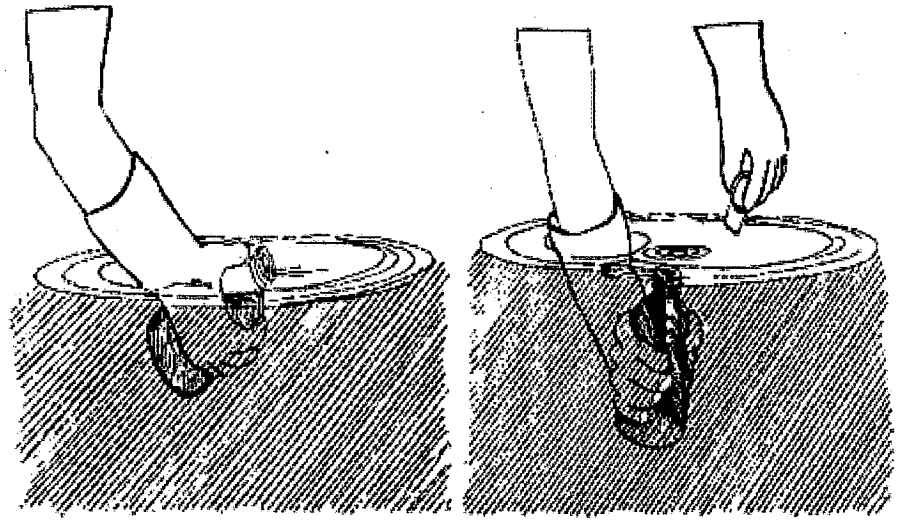

Figure 5.7

Taking a water sample for DO analysis
Point the bottle downstream and fill gradually. Cap underwater when full.

Using a DO Meter

If you are using a dissolved oxygen meter, be sure that it is calibrated immediately prior to use. Check the cable connection between the probe and the meter. Make sure that the probe is filled with electrolyte solution, that the membrane has no wrinkles, and that there are no bubbles trapped on the face of the membrane. You can do a field check of the meter's accuracy by calibrating it in saturated air according to th e manufacturer's instructions. Or, you can measure a water sample that is saturated with oxygen, as follows. (NOTE: You can also use this procedure for testing the accuracy of the Winkler method.)

1. Fill a l-liter beaker or bucket of tap water. (You may want to bring a gallon jug with water in it for this purpose.) Mark the bottle number as "tap" on the lab sheet.
2. Pour this water back and forth into another beaker 10 times to saturate the water with oxygen.
3. Use the meter to measure the water temperature and record it in the water temperature column on the field data sheet.
4. Find the water temperature of your "tap" sample in Table 5.3. Use the meter to compare the dissolved oxygen concentration of your sample with the maximum concentration at that temperature in the table. Your sample should be within 0.5 mg/L. If it is not, repeat the check and if there is still an error, check the meter's batteries and follow the troubleshooting procedures in the manufacturer's manual.

Once the meter is turned on, allow 15 minute equilibration before calibrating. After calibration, do not turn the meter off until the sample is analyzed. Once you have verified that the meter is working properly, you are ready to measure the DO levels at the sampling site. You might need an extension pole (this can be as simple as a piece of wood) to get the probe to the proper sampling point. Simply secure the probe to the end of the extension pole. A golfer's ball retriever works well because it is collapsible and easy to transport. To use the probe, proceed as follows:

1. Place the probe in the stream below the surface.
2. Set the meter to measure temperature, and allow the temperature reading to stabilize. Record the temperature on the field data sheet.

3. Switch the meter to read dissolved oxygen.
4. Record the dissolved oxygen level on the field data sheet.

TASK 4 Analyze the samples

Three types of titration apparatus can be used with the Winkler method: droppers, digital titrators, and burets. The dropper and digital titrator are suited for field use. The buret is more conveniently used in the lab (Fig. 5.8) Volunteer programs are most likely to use the dropper or digital titrator. For titration with a dropper or syringe, which is relatively simple, follow the manufacturer's instructions. The following procedure is for using a digital titrator to determine the quantity of dissolved oxygen in a fixed sample:

1. Select a sample volume and sodium thiosulfate titration cartridge for the digital titrator corresponding to the expected dissolved oxygen concentration according to Table 5.4. In most cases, you will use the 0.2 N cartridge and the 100-mL sample volume.
2. Insert a clean delivery tube into the titration cartridge.
3. Attach the cartridge to the titrator body.
4. Hold the titrator with the cartridge tip up. Turn the delivery knob to eject air and a few drops of titrant. Reset the counter to 0 and wipe the tip.
5. Use a graduated cylinder to measure the sample volume (from the "fixed" sample in the 300-mL BOD bottle) according to Table 5.4.
6. Transfer the sample into a 250-mL Erlenmeyer flask, and place the flask on a magnetic stirrer with a stir bar. If you are in the field, you can manually swirl the flask to mix.
7. Place the delivery tube tip into the solution and turn the stirrer on to stir the sample while you're turning the delivery knob.
8. Titrate to a pale yellow color.
9. Add two dropperfuls of starch indicator solution and swirl to mix. A strong blue color will develop.
10. Continue to titrate until the sample is clear. Record the number of digits required. (The color might reappear after standing a few minutes, but this is not a cause for concern. The "first" disappearance of the blue color is considered the endpoint.)
11. Calculate mg/L of DO = digits required X digit multiplier (from Table 5.4).
12. Record the results in the appropriate column of the data sheet.

Some water quality standards are expressed in terms of percent saturation. To calculate percent saturation of the sample:

1. Find the temperature of your water sample as measured in the field.
2. Find the maximum concentration of your sample at that temperature as given in Table 5.3.
3. Calculate the percent saturation, by dividing your actual dissolved oxygen by the maximum concentration at the sample temperature.
4. Record the percent saturation in the appropriate column on the data sheet.

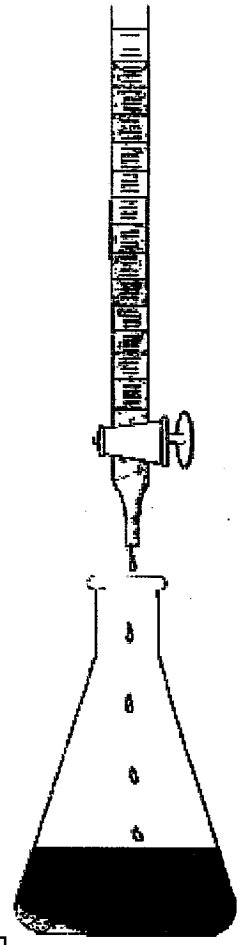

Figure 5.8

Titrating a DO sample using a buret

TASK 5 Return the samples and the field data sheets to the lab/drop-off point

If you are using the Winkler method and delivering the samples to a lab for titration, double-check to make sure that you have recorded the necessary information for each site on the field data

Table 5.4

Sample volume selection and

Expected Range	Sample Volume	Titration Cartridge	Digit Multiplier
1-5 mg/L	200 mL	0.2 N	0.01

sheet, especially the bottle number and corresponding site number and the times the samples were collected. Deliver your samples and field data sheets to the lab. If you have already obtained the dissolved oxygen results in the field, send the data sheets to your sampling coordinator.

2-10 mg/L	100 mL	0.2 N	0.02	**corresponding values for Winkler titration**
10+ mg/L	200 mL	2.0 N	0.10	

What is biochemical oxygen demand and why is it important?

Biochemical oxygen demand, or BOD, measures the amount of oxygen consumed by microorganisms in decomposing organic matter in stream water. BOD also measures the chemical oxidation of inorganic matter (i.e., the extraction of oxygen from water via chemical reaction). A test is used to measure the amount of oxygen consumed by these organisms during a specified period of time (usually 5 days at 20 C). The rate of oxygen consumption in a stream is affected by a number of variables: temperature, pH, the presence of certain kinds of microorganisms, and the type of organic and inorganic material in the water.

BOD directly affects the amount of dissolved oxygen in rivers and streams. The greater the BOD, the more rapidly oxygen is depleted in the stream. This means less oxygen is available to higher forms of aquatic life. The consequences of high BOD are the same as those for low dissolved oxygen: aquatic organisms become stressed, suffocate, and die.

Sources of BOD include leaves and woody debris; dead plants and animals; animal manure; effluents from pulp and paper mills, wastewater treatment plants, feedlots, and food-processing plants; failing septic systems; and urban stormwater runoff.

Sampling Considerations

BOD is affected by the same factors that affect dissolved oxygen (see above). Aeration of stream water by rapids and waterfalls, for example will accelerate the decomposition of organic and inorganic material. Therefore, BOD levels at a sampling site with slower, deeper waters might be higher for a given volume of organic and inorganic material than the levels for a similar site in highly aerated waters.

Chlorine can also affect BOD measurement by inhibiting or killing the microorganisms that decompose the organic and inorganic matter in a sample. If you are sampling in chlorinated waters, such as those below the effluent from a sewage treatment plant, it is necessary to neutralize the chlorine with sodium thiosulfate. (See APHA, 1992.)

BOD measurement requires taking two samples at each site. One is tested immediately for dissolved oxygen, and the second is incubated in the dark at 20 C for 5 days and then tested for the amount of dissolved oxygen remaining. The difference in oxygen levels between the first test and the second test, in milligrams per liter (mg/L), is the amount of BOD. This represents the amount of oxygen consumed by microorganisms to break down the organic matter present in the sample bottle during the incubation period. Because of the 5-day incubation, the tests should be conducted in a laboratory.

Sometimes by the end of the 5-day incubation period the dissolved oxygen level is zero. This is especially true for rivers and streams with a lot of organic pollution. Since it is not known when the zero point was reached, it is not possible to tell what the BOD level is. In this case it is necessary to dilute the original sample by a factor that results in a final dissolved oxygen level of at least 2 mg/L. Special dilution water should be used for the dilutions. (See APHA, 1992.)

It takes some experimentation to determine the appropriate dilution factor for a particular sampling site. The final result is the difference in dissolved oxygen between the first measurement and the second after multiplying the second result by the dilution factor. More details are provided in the following section.

How to Collect and Analyze Samples

The procedures for collecting samples for BOD testing consist of the same steps described for sampling for dissolved oxygen (see above), with one important difference. At each site a second sample is collected in a BOD bottle and delivered to the lab for DO testing after the 5-day incubation period. Follow the same steps used for measuring dissolved oxygen with these additional considerations:

- Make sure you have two BOD bottles for each site you will sample. The bottles should be black to prevent photosynthesis. You can wrap a clear bottle with black electrician's tape if you do not have a bottle with black or brown glass.
- Label the second bottle (the one to be incubated) clearly so that it will not be mistaken for the first bottle.
- Be sure to record the information for the second bottle on the field data sheet.

The first bottle should be analyzed just prior to storing the second sample bottle in the dark for 5 days at 20 C. After this time, the second bottle is tested for dissolved oxygen using the same method that was used for the first bottle. The BOD i s expressed in milligrams per liter of DO using the following equation:

DO (mg/L) of first bottle
- DO (mg/L) of second bottle
= BOD (mg/L)

References

APHA. 1992. *Standard methods for the examination of water and wastewater.* 18th ed. American Public Health Association, Washington, DC.

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