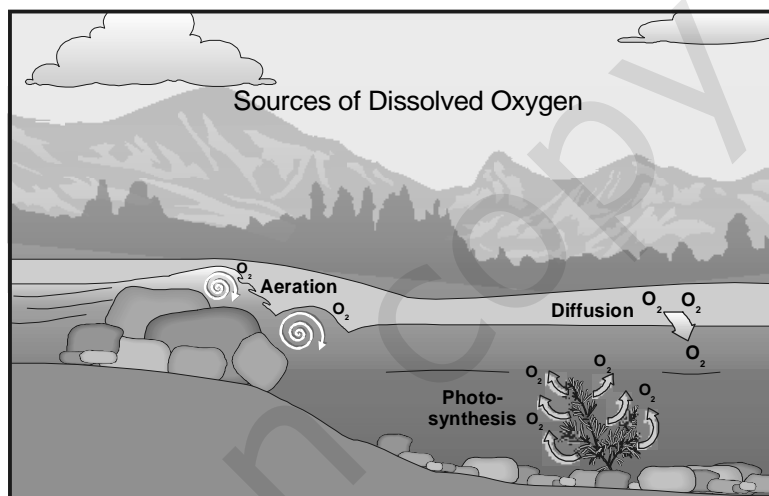


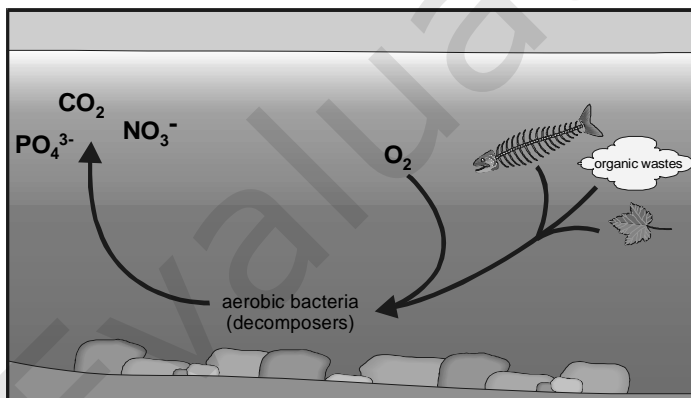
# Biochemical Oxygen Demand

## INTRODUCTION

Oxygen available to aquatic organisms is found in the form of *dissolved oxygen*. Oxygen gas is dissolved in a stream through aeration, diffusion from the atmosphere, and photosynthesis of aquatic plants and algae. Plants and animals in the stream consume oxygen in order to produce energy through respiration. In a healthy stream, oxygen is replenished faster than it is used by aquatic organisms. In some streams, aerobic bacteria decompose such a large volume of organic material that oxygen is depleted from the stream faster than it can be replaced. The resulting decrease in dissolved oxygen is known as the *Biochemical Oxygen Demand* (BOD).



When it rains, organic material found in the soil is transported in the rainwater to streams and rivers. Additional organic material accumulates in the stream when aquatic organisms die. Bacteria and other microorganisms decompose this organic material. In a healthy body of water, this process has only a slight impact on dissolved oxygen levels. It serves to release vital



nutrients, such as nitrates and phosphates, which stimulate algae and aquatic plant growth. If the amount of decomposing organic material is too high, dissolved oxygen levels can be severely reduced. In a body of water with large amounts of decaying organic material the dissolved oxygen levels may drop by 90%—this would represent a high BOD. In a mountain stream with low levels of decaying organic material, the dissolved oxygen levels may drop by only 10% or 20%—a low BOD.

Organic materials, such as leaves, fallen trees, fish carcasses, and animal waste, end up in the water naturally and are important in the recycling of nutrients throughout the ecosystem. Organic materials that enter the water as a result of human impact can be considered sources of pollution.

## Expected Levels

BOD levels are dependent on the body of water being tested. Shallow, slow-moving waters, such as ponds and wetlands, will often have large amounts of organic material in the water and high

BOD levels. A water sample from a pond could have an initial dissolved oxygen reading of 9.5 mg/L. After the five-day incubation period, the dissolved oxygen could be down to 1 mg/L resulting in a high BOD level of 8.5 mg/L. In contrast, a water sample collected from a cold mountain stream with an initial dissolved oxygen reading of 11 mg/L may have decreased to 9 mg/L after incubation, resulting in a BOD of only 2 mg/L. Use Table 1 as a rough guide for the data you gather<sup>1</sup>.

BOD Level (mg/L)	Status
1–2 mg/L	Clean water with little organic waster.
3–5 mg/L	Moderately clean water with some organic waste.
6–9 mg/L	Lots of organic material and many bacteria.
>10 mg/L	Very poor water quality. Large amounts of organic material in the water.

## Summary of Methods

Included in this test are the procedures for High and Low BOD levels. Decide beforehand, based on expected BOD levels (see Table 1), which procedure is appropriate for the water you are testing. Only one of the two tests should be performed.

### Method 1: Low BOD Levels (0–6 mg/L)

BOD is calculated from two separate dissolved oxygen measurements made using the Dissolved Oxygen Probe. The initial dissolved oxygen reading is taken at the sampling site using the procedures outlined in Test 5. Using a light-free sample bottle, a water sample is collected at the same site. The sample is transported back to the lab and incubated at 20°C for a total of five days. After five days, the incubated sample is tested for dissolved oxygen. The oxygen reading at the end of the five days is subtracted from the initial reading. The resulting value is the BOD level.

### Method 2: High BOD Levels (> 6 mg/L)

This method is recommended when testing stagnant or polluted waters, in which all of the dissolved oxygen may be consumed before the end of the 5-day period. The initial dissolved oxygen test, sampling, storage and incubation, are performed in the same manner as found in Method 1. Differences for Method 2 are:

- Five water samples are collected.
- A sample is tested for dissolved oxygen every 24 hours for five days.
- If, before the fifth day, the dissolved oxygen concentration falls below 4.0 mg/L, oxygen is added to the remaining samples by aeration.
- Add each bottle's change in dissolved oxygen concentration to obtain the BOD value.

<sup>1</sup> Table 1 is from the Student Watershed Research Project manual, 3<sup>rd</sup> Edition 1996.

**Method 1: LOW BOD (0–6 mg/L)****Materials Checklist**

- |   |   |
|---|---|
| ___ computer                            | ___ 5 BOD bottles                         |
| ___ Vernier computer interface          | ___ aluminum foil                         |
| ___ Logger <i>Pro</i>                   | ___ 100% calibration bottle               |
| ___ Vernier Dissolved Oxygen Probe      | ___ wash bottle with distilled water      |
| ___ D.O. Electrode Filling Solution     | ___ tissues or paper towels               |
| ___ Sodium Sulfite Calibration Solution | ___ pipet                                 |
| ___ 250 mL beaker                       | ___ small plastic or paper cup (optional) |

**Testing Procedure****Day 0**

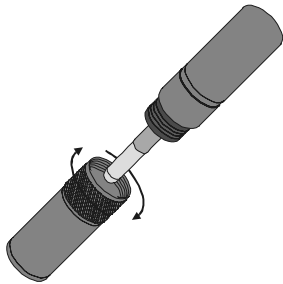
1. Obtain an initial dissolved oxygen reading at the site you are testing for BOD. If another student group is performing the dissolved oxygen test outlined in Test 5, then copy their dissolved oxygen readings onto the BOD Data & Calculations sheet under the heading of Initial Dissolved Oxygen. If no one is taking an initial dissolved oxygen reading at this site, you must perform the dissolved oxygen test prior to beginning this test.
2. Collect three water samples for the BOD test at the same location the initial dissolved oxygen reading was measured. Using the glass BOD sample bottles, submerge each sample bottle 10 cm below the water's surface and keep it there for 1 minute. When a minute has elapsed, the bottle should be void of any air bubbles and completely full. Place the bottle lid back on the bottle and screw the lid down tight while still submerged. Each bottle should be completely covered with aluminum foil or black tape to block out any light.
3. If the time between collection of samples and incubation of samples is greater than 30 minutes, place the filled bottles in an ice chest until they can be placed in an incubator. If the time is less than 30 minutes, simply keep the filled bottles out of direct sunlight.
4. Upon returning to the lab, place the BOD bottles in an incubator or dark closet at about 20°C. The bottles should remain in the incubator or closet for five days until you are ready to perform the final dissolved oxygen measurement in the Day-5 procedure below.

**Day 5**

When five days have passed, perform Steps 5–12 to test the incubated samples for dissolved oxygen. If possible, try to test the samples at roughly the same time of day they were collected.

5. Position the computer safely away from the water. Keep water away from the computer at all times.
6. Plug the Dissolved Oxygen Probe into Channel 1 of the Vernier interface.
7. You are now ready to prepare the Dissolved Oxygen Probe for use.
  - a. Remove the blue protective cap if it is still on the tip of the probe.
  - b. Unscrew the membrane cap from the tip of the probe.

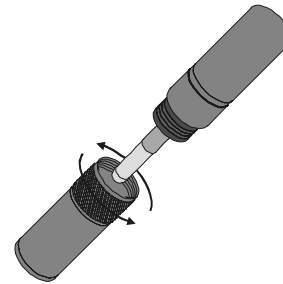
- c. Using a pipet, fill the membrane cap with 1 mL of D.O. Electrode Filling Solution.
- d. Carefully thread the membrane cap back onto the electrode.
- e. Place the probe into a container of water.



Remove membrane cap



Add electrode filling solution

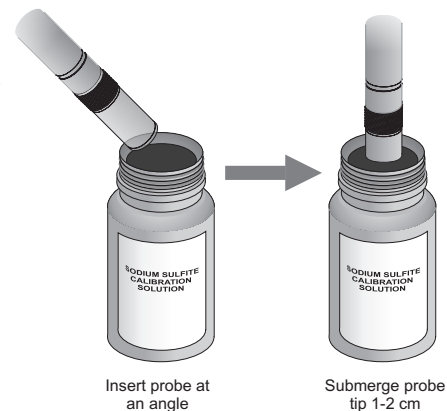


Replace membrane cap

8. Prepare the computer for data collection by opening the file “06 BOD” from the *Water Quality with Vernier* folder of *LoggerPro*.
9. It is necessary to warm up the Dissolved Oxygen Probe for 5–10 minutes before taking readings. To warm up the probe, leave it connected to the interface, with *Logger Pro* running, for 5–10 minutes. The probe must stay connected at all times to keep it warmed up. If disconnected for a few minutes, it will be necessary to warm up the probe again.
10. You are now ready to calibrate the Dissolved Oxygen Probe.
  - If your instructor directs you to use the calibration stored in the experiment file, then proceed to Step 11.
  - If your instructor directs you to perform a new calibration for the Dissolved Oxygen Probe, follow this procedure.

#### Zero-Oxygen Calibration Point

- a. Choose Calibrate ► CH1: Dissolved Oxygen (mg/L) from the Experiment menu and then click .
- b. Remove the probe from the water bath and place the tip of the probe into the Sodium Sulfite Calibration Solution. **Important:** No air bubbles can be trapped below the tip of the probe or the probe will sense an inaccurate dissolved oxygen level. If the voltage does not rapidly decrease, tap the side of the bottle with the probe to dislodge the bubble. The readings should be in the 0.2 to 0.5 V range.
- c. Type **0** (the value in mg/L) in the edit box.
- d. When the displayed voltage reading for Reading 1 stabilizes, click .

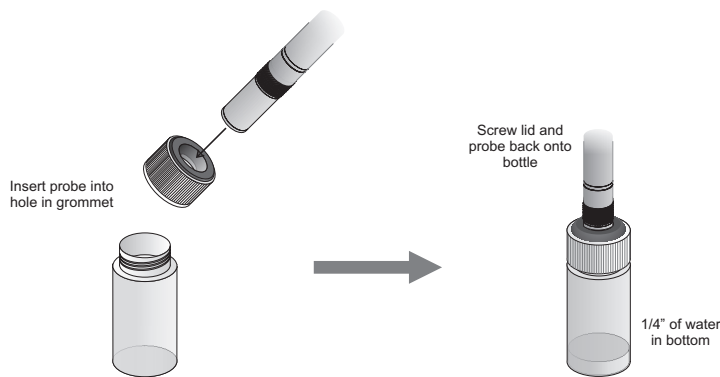


Insert probe at an angle

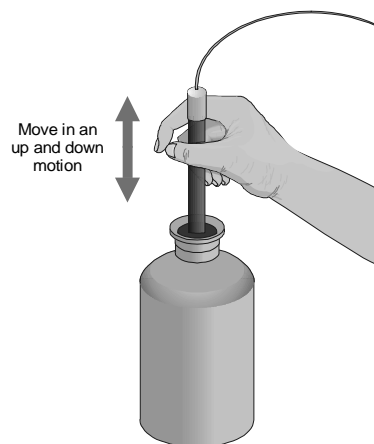
Submerge probe tip 1-2 cm

#### Saturated D.O. Calibration Point

- e. Rinse the probe with distilled water.
- f. Unscrew the lid of the calibration bottle provided with the probe. Slide the lid and the grommet about 1/2 inch onto the probe body.



- g. Add water to the bottle to a depth of about 1/4 inch and screw the bottle into the cap, as shown. Keep the probe in this position for about a minute. **Important:** Do not touch the membrane or get it wet during this step.
  - h. Type the correct saturated dissolved oxygen value (in mg/L) from Table 3 of the Dissolved Oxygen chapter (for example, **8.66**) using the current barometric pressure and air temperature values. If you do not have the current air pressure, use Table 4 of the Dissolved Oxygen chapter to determine the approximate air pressure at your altitude.
  - i. When the displayed voltage reading for Reading 2 stabilizes (readings should be above 2.0 V), click  and then click .
11. Remove the water samples from the incubator.
12. You are now ready to collect dissolved oxygen concentration data.
- a. Submerge the probe tip in the BOD bottle. Gently move the probe in and up-and-down motion, while keeping the tip in the water at all times.
  - b. Click  to begin data collection.
  - c. Click  to begin a 10 s sampling run. **Important:** Leave the probe tip submerged for the 10 seconds that data is being collected.
  - d. When the sampling run is complete, stop data collection and record the dissolved oxygen value on the Data & Calculations sheet as the final 5 day DO reading.
13. Return to Step 12 to obtain a reading for the other two samples. When all readings have been taken, rinse the tip of the probe and secure it in the calibration bottle filled with water.



## DATA & CALCULATIONS

### Low BOD Levels (0–6 mg/L)

Stream or lake: \_\_\_\_\_ Time of day: \_\_\_\_\_

Site name: \_\_\_\_\_ Student name: \_\_\_\_\_

Site number: \_\_\_\_\_ Student name: \_\_\_\_\_

Date: \_\_\_\_\_ Student name: \_\_\_\_\_

	A	B	C
Sample	Initial dissolved oxygen (mg/L)	Final dissolved oxygen (mg/L)	BOD (mg/L)
Example	10.8 mg/L	6.7 mg/L	4.1 mg/L
1			
2			
3			
Average			

Column Procedure:

- A. Record dissolved oxygen reading from DO test performed at the sample site.
- B. Record dissolved oxygen reading from DO test after incubation of sample for five days.
- C. Calculate  $BOD = A - B = C$

Field Observations (e.g., weather, geography, vegetation along stream) \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Test Completed: \_\_\_\_\_ Date: \_\_\_\_\_

**Method 2: HIGH BOD (>6 mg/L)****Materials Checklist**

- |   |   |
|---|---|
| ___ computer                            | ___ 5 BOD bottles                         |
| ___ Vernier computer interface          | ___ aluminum foil                         |
| ___ Logger <i>Pro</i>                   | ___ 100% calibration bottle               |
| ___ Vernier Dissolved Oxygen Probe      | ___ wash bottle with distilled water      |
| ___ D.O. Electrode Filling Solution     | ___ tissues or paper towels               |
| ___ Sodium Sulfite Calibration Solution | ___ pipet                                 |
| ___ 250 mL beaker                       | ___ small plastic or paper cup (optional) |

**Testing Procedure****Day 0**

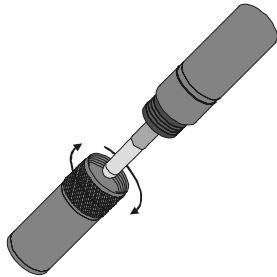
1. Obtain an initial dissolved oxygen reading at the site you are testing for BOD. If another student group is performing the dissolved oxygen test outlined in Test 5, then copy their dissolved oxygen readings onto the BOD Data & Calculations sheet as the Initial and Final Dissolved Oxygen reading for Day 0. If no one is taking an initial dissolved oxygen reading at this site, you must perform the dissolved oxygen test prior to beginning this test.
2. Collect five water samples for the BOD test at the same location the initial dissolved oxygen reading was measured. Using the glass BOD sample bottles, submerge each sample bottle 10 cm below the water's surface and keep it there for 1 minute. When one minute has elapsed, the bottle should be void of any air bubbles and completely full. Place the bottle lid back on the bottle and screw the lid down tight while still submerged. Each bottle should be completely covered with aluminum foil or black tape to block out any light. Using tape, label the bottles 1 through 5.
3. If the time between collection of samples and incubation of samples is greater than 30 minutes, place the filled bottles in an ice chest until they can be placed in an incubator. If the time is less than 30 minutes, simply keep the filled bottles out of direct sunlight.
4. Upon returning to the lab, place the BOD bottles in an incubator or dark closet set at 20°C. The bottles should remain in the incubator, or closet until you are ready to perform the first dissolved oxygen measurement in the Day 1–5 procedure below.

**Day 1–5**

When 24 hours have passed, perform Steps 5–13 to test an incubated sample for dissolved oxygen. The sample should be tested at approximately the same time of day the initial dissolved oxygen measurement was made.

5. Position the computer safely away from the water. Keep water away from the computer at all times.
6. Plug the Dissolved Oxygen Probe into Channel 1 of the Vernier interface.
7. You are now ready to prepare the Dissolved Oxygen Probe for use.
  - a. Remove the blue protective cap if it is still on the tip of the probe.

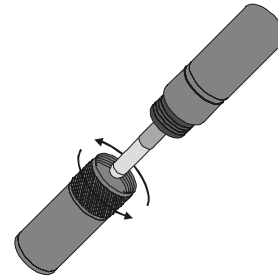
- b. Unscrew the membrane cap from the tip of the probe.
- c. Using a pipet, fill the membrane cap with 1 mL of D.O. Electrode Filling Solution.
- d. Carefully thread the membrane cap back onto the electrode.
- e. Place the probe into a container of water.



Remove membrane cap



Add electrode filling solution

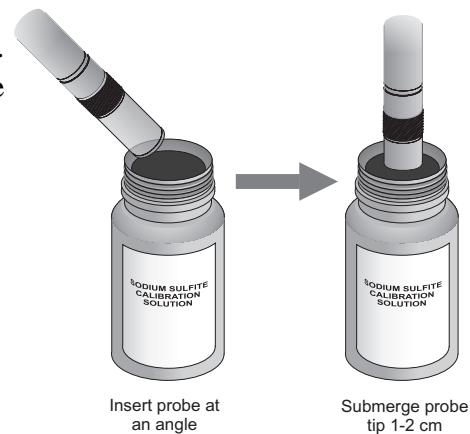


Replace membrane cap

8. Prepare the computer for data collection by opening the file “06 BOD” from the *Water Quality with Vernier* folder of *LoggerPro*.
9. It is necessary to warm up the Dissolved Oxygen Probe for 5–10 minutes before taking readings. To warm up the probe, leave it connected to the interface, with *Logger Pro* running, for 5–10 minutes. The probe must stay connected at all times to keep it warmed up. If disconnected for a few minutes, it will be necessary to warm up the probe again.
10. You are now ready to calibrate the Dissolved Oxygen Probe.
  - If your instructor directs you to use the calibration stored in the experiment file, then proceed to Step 11.
  - If your instructor directs you to perform a new calibration for the Dissolved Oxygen Probe, follow this procedure.

#### Zero-Oxygen Calibration Point

- a. Choose Calibrate ► CH1: Dissolved Oxygen (mg/L) from the Experiment menu and then click .
- b. Remove the probe from the water bath and place the tip of the probe into the Sodium Sulfite Calibration Solution. **Important:** No air bubbles can be trapped below the tip of the probe or the probe will sense an inaccurate dissolved oxygen level. If the voltage does not rapidly decrease, tap the side of the bottle with the probe to dislodge the bubble. The readings should be in the 0.2 to 0.5 V range.
- c. Type 0 (the value in mg/L) in the edit box.
- d. When the displayed voltage reading for Reading 1 stabilizes, click .



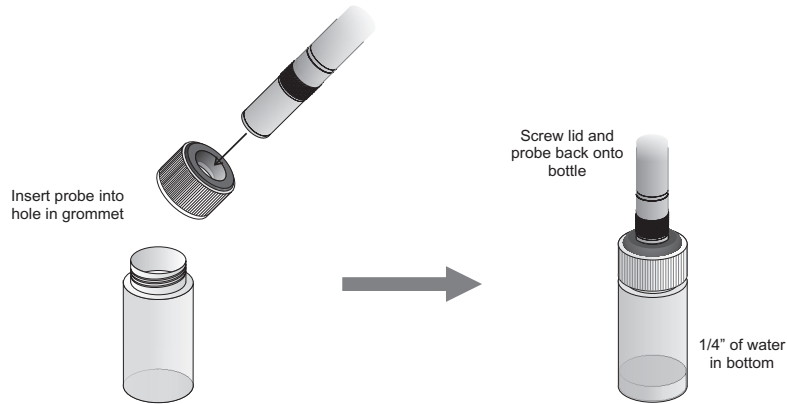
Insert probe at an angle

Submerge probe tip 1-2 cm

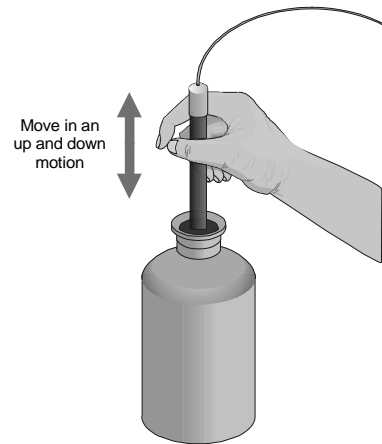
#### Saturated D.O. Calibration Point

- e. Rinse the probe with distilled water.
- f. Unscrew the lid of the calibration bottle provided with the probe. Slide the lid and the grommet about 1/2 inch onto the probe body.



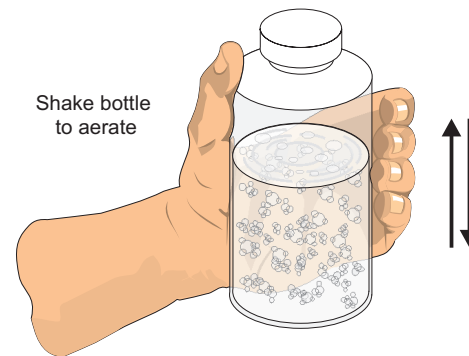
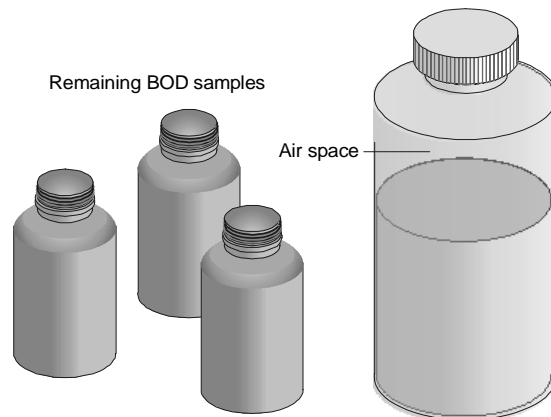


- g. Add water to the bottle to a depth of about 1/4 inch and screw the bottle into the cap, as shown. Keep the probe in this position for about a minute. **Important:** Do not touch the membrane or get it wet during this step.
  - h. Type the correct saturated dissolved oxygen value (in mg/L) from Table 3 of the Dissolved Oxygen chapter (for example, **8.66**) using the current barometric pressure and air temperature values. If you do not have the current air pressure, use Table 4 of the Dissolved Oxygen chapter to determine the approximate air pressure at your altitude.
  - i. When the displayed voltage reading for Input 1 stabilizes (readings should be above 2.0 V), click  and then click .
11. Remove a water sample from the incubator.
12. You are now ready to collect dissolved oxygen concentration data.
- a. Submerge the probe tip in the BOD bottle. Gently move the probe in and up-and-down motion, while keeping the tip in the water at all times.
    - a. Click  to begin data collection.
    - b. Click  to begin a 10 s sampling run. **Important:** Leave the probe tip submerged for the 10 seconds that data is being collected.
    - c. When the sampling run is complete, stop data collection and record the mean dissolved oxygen concentration value on the Data & Calculations sheet in the row for the day you are testing.



13. If the dissolved oxygen level falls below 4.0 mg/L before Day 5 it will be necessary to aerate the remaining water samples.

- a. Pour all remaining BOD water samples into a clean container or bottle that can be closed with a lid. Be sure to leave several inches of airspace in the top of the container. If this is not possible, you will need to find a larger container.
- b. Vigorously shake the closed container for one minute. Uncap for 30 seconds then close again. Shake for another minute and then pour the water back into the BOD bottles. This should sufficiently aerate the water, bringing the dissolved oxygen levels well above the 4.0 mg/L mark.
- c. Measure the dissolved oxygen of one of the BOD bottles as described in Step 12. Close all of the BOD bottles, making sure that they are all brim full with no air space visible within.
- d. Record the new dissolved-oxygen reading as the Initial Dissolved Oxygen for the next day on the Data & Calculations sheet. For example, if you are testing on Day 3, then record the new dissolved oxygen reading under the Initial Dissolved Oxygen column heading of Day 4.



14. In 24 hours, repeat Steps 5–13 to determine the dissolved-oxygen concentration of the next sample bottle. Continue testing a sample each day until all five samples have been tested. Calculate BOD as described on the Data & Calculations sheet.

*Biochemical Oxygen Demand*

**DATA & CALCULATIONS**

**High BOD Levels (>6 mg/L)**

Stream or lake: \_\_\_\_\_ Time of day: \_\_\_\_\_

Site name: \_\_\_\_\_ Student name: \_\_\_\_\_

Site number: \_\_\_\_\_ Student name: \_\_\_\_\_

Date: \_\_\_\_\_ Student name: \_\_\_\_\_

A		B	C	D
Aeration √	Day	Initial dissolved oxygen (mg/L)	Final dissolved oxygen (mg/L)	ΔDO (mg/L)
	Example	10.2 mg/L	7.7 mg/L	2.5 mg/L
	Example	7.7 mg/L	5.1 mg/L	2.6 mg/L
	Day 0			
	Day 1			
	Day 2			
	Day 3			
	Day 4			
	Day 5			
		<b>E</b>		BOD

Column Procedure:

- A. If it was necessary to aerate the sample, place a check in column A.
- B. Record final dissolved-oxygen reading from previous 24 hour test or first initial reading.
- C. Record dissolved-oxygen reading from DO test performed on current day.
- D. Calculate  $\Delta DO = B - C = D$
- E. BOD = Sum of column D (day 0 through 5).

Field Observations (e.g., weather, geography, vegetation along stream) \_\_\_\_\_

Test Completed: \_\_\_\_\_ Date: \_\_\_\_\_

# Vernier Lab Safety Instructions Disclaimer

**THIS IS AN EVALUATION COPY OF THE VERNIER STUDENT LAB.**

This copy does not include:

- Safety information
- Essential instructor background information
- Directions for preparing solutions
- Important tips for successfully doing these labs

The complete *Water Quality with Vernier* lab manual includes 16 water quality tests and essential teacher information. The full lab book is available for purchase at:

<http://www.vernier.com/cmat/wqv.html>



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