DISSOLVED OXYGEN (DO or Dissolved O_2)

Among the most important variables affecting stream health is the concentration of dissolved oxygen present in the water. It is essential to numerous life forms and is readily affected by many human activities. In this activity, students will measure the amount of dissolved oxygen in a water sample from the Huron River watershed.

Although the pre and post discussions are necessary to provide important information about each topic, it is the activity that is most vital to this unit. Be sure to allow plenty of time to complete the activity.

Pre-Activity Discussion (Answers can be found in the Background Information section below)

Before beginning the experiment ask the students: 1) What is dissolved oxygen and why is it important? How does it differ from the combined oxygen in H_2O? 2) What are some natural sources of dissolved oxygen in water? 3) What natural phenomena affect the level of dissolved oxygen in stream water? 4) What human activities affect the level of dissolved oxygen in stream water?

The Activity

Equipment

--Hach field test kit for dissolved oxygen: Azide-modified Winkler method
--Scissors or small cutting pliers to open powder pillows in Hach kit
--Small table
--Long-handled dipper (if you need to sample water from a steep bank)
--Display board
--Data sheets and clipboard
--Wastewater jug for disposal of spent solutions
- -Safety glasses

The Process

You will be using a Hach portable field test kit for this measurement. The procedure is below and is easy to follow if you do each step carefully. But the chemistry, explained in the following paragraph, is complicated.

In brief, you will be removing interfering NO_2 (nitrite ion) from the water sample with alkaline sodium azide. Manganous sulfate ($MnSO_4$) and potassium iodide (KI) are then added to the sample. A white precipitate of manganous hydroxide ($MnOH_2$) is formed which rapidly reacts with any dissolved oxygen present to form brown manganese dioxide (MnO_2). The solution is then made acid by adding sulfamic acid (H_3NSO_3). In this acidic solution the manganese dioxide reacts with iodide ion (from the potassium iodide added earlier) to form an amount of iodine (I_2) chemically equivalent to the amount of dissolved oxygen originally present in the sample. Lastly, the released I_2 is titrated using a carefully measured amount of sodium thiosulfate ($Na_2S_2O_3$) solution. This converts the brown I_2 to colorless I^- leaving a colorless solution. The amount of sodium thiosulfate needed to turn the water colorless is proportional to the concentration of dissolved oxygen in the original sample.

Toxicity and Disposal Information for Activity Leaders and Students

These chemicals are toxic (some quite toxic) and very irritating to the eyes. Wear safety goggles, avoid spills, DO NOT TASTE ANY OF THE CHEMICALS, and rinse your hands well with water after completing the activity. If you get any of these chemicals in an eye, wash the eye with clean water (such as drinking water) for 5 minutes and then seek medical attention at once. Dispose of liquid wastes from the activity in the jug provided for later disposal in a sink or toilet.

Set-up

Select a site with space for a small table and easy access to the stream. You will need to be upstream from activities that may cause a lot of turbidity (macroinvertebrate collection, stream speed). The water should be deep enough to allow you to hold the sample bottle 6-12 inches under the surface of the water while filling it.

Student Roles

In order to keep the students involved, give as many as possible a task. One student can handle the data sheet. Another can fetch water samples and dispose of treated water in a container. One student can read the steps of the procedure to the group. A simplified version of the steps is included on a separate page for this purpose. It follows the background information. Be sure to print it out. Lastly, one or two students can wear latex gloves and add the chemicals to the water sample. While the students are following the steps below, you can give an explanation as to what is happening.

Procedure (Technical information is in parentheses)

1. Fill the glass-stoppered bottle with sample water, allowing the water to overflow for a minute and making sure there are no bubbles in the bottle. If possible, fill this bottle by holding it 6-12 inches below the surface of the water in the stream.

2. Tip the bottle slightly and stopper it WITHOUT trapping any air bubbles. The concentration of oxygen in air is roughly 30 times its concentration in water so a small air bubble can throw off your results making them high.

3. Remove stopper and add the contents of a DO 1 reagent envelope. (This envelope contains sodium azide that will prevent nitrite interference, and sodium hydroxide to make the solution alkaline.) Also add a DO reagent 2 envelope. (This envelope contains manganous sulfate and potassium iodide.) Stopper the bottle carefully to avoid trapping any bubbles.

4. Invert the bottle several times to dissolve the powders. A precipitate (floc) will form. It will be brown if oxygen is present. (The floc is a mixture of white manganous hydroxide and dark brown manganese dioxide.) Let the floc settle to about half the bottle volume.

5. Invert the bottle once more to mix and again let the floc settle to about half the bottle volume. This gives time for the reaction between manganous hydroxide and dissolved oxygen to go to completion.

6. Remove the stopper and add the contents of one DO 3 reagent powder pillow. (This pillow contains sulfamic acid. It is much safer than sulfuric acid and just as effective).

Invert the bottle several times to mix. The sample will turn clear yellow-brown if oxygen was present.

7. Add one full measuring tube- the small round tube- of the sample to the square bottle.

8. Add sodium thiosulfate solution one drop at a time to the square bottle, swirling it after the addition of each drop and counting the number of drops added. Stop when the color of the solution changes from yellow to colorless. This is easiest to see if done on a white background. A piece of white paper is fine. (The thiosulfate reacts with the colored iodine to produce colorless iodide ion.)

9. The number of drops of thiosulfate solution added to the sample is equal to the DO concentration in the water in mg/L. (milligrams per liter)

10. Optional: If you wish, you can check your accuracy by discarding the solution in the square bottle, rinsing it, and then adding **two** full measuring tubes of sample from the glass-stoppered bottle to the square bottle. Then add sodium thiosulfate one drop at a time as in Step 8, swirling and counting drops as before until the solution becomes colorless. Here the DO concentration is **half** the number of drops added.

11. If time permits, rotate assignments among the students and make a second run, repeating Steps 1 through 9 above.

Examining the Results

Compare the results of the testing to the chart below.

Table 1: Minimum DO requirements for some aquatic organisms

Organism	Minimum D.O. requirement (mg/L)
Trout	6.5
Smallmouth bass	6.5
Caddisfly larvae	4.0
Mayfly larvae	4.0
Catfish	2.5
Carp	2.0
Mosquito larvae	1.0

Note: DO requirements for fish depend on a number of factors, so the numbers in Table 1 are not precise and other sources may give slightly different figures. Nevertheless Table 1 provides useful approximate information about the DO needs of aquatic life.

Post-activity discussion questions:

1) What do our results tell us about the stream water? 2) Are there any conditions nearby that might affect the amount of dissolved oxygen concentration in the portion of the stream being sampled? 3) The chart below shows how the ability of water to hold dissolved oxygen decreases as water temperature rises. What are some things that can

cause water temperatures to rise? What can we do to prevent the warming of stream waters? 4) What other things can be done to protect against low levels of dissolved oxygen?

Table 2: Oxygen solubility in water at 1 atmosphere (760 mm Hg) pressure of air

Temperature ^oC	Temperature ^oF	Oxygen solubility: mg/L
10	50	11.28
20	68	9.09
30	86	7.56
40	104	6.41

Background Information

Dissolved oxygen is essential for fish and benthic macroinvertebrates to live. When there is not enough oxygen, the food web for the whole ecosystem is affected. It must be remembered that the oxygen that hooks up with hydrogen to make water is not available for respiration. Although the concentration of oxygen in the air is quite high, O_2 is not very soluble in water. At a pressure of one atmosphere (atm) of air, the oxygen concentration in water at room temperature is about 8.2 mg/L or 8.2 parts per million (ppm) at saturation level.

Levels of DO are affected by discharges from industrial facilities and water treatment plants. They are also affected by water released from dams. DO concentrations in Michigan waters are monitored by the Michigan Department of Natural Resources. Violations are investigated and corrective action is taken. According to the laws of the State of Michigan, the minimum permissible DO in most Michigan streams is 5.0 mg/L. This is enough to support most aquatic life (but not trout or small-mouth bass) however the margin is not large. Another reason that the DNR monitors DO levels is to determine where to stock fish and the kind of fish to stock.

Dissolved oxygen can enter stream or lake water in many ways. Below are some common sources.

1) Diffusion from the atmosphere
2) Aeration as water moves over rocks and debris, riffles, rapids, waterfalls, etc.
3) Aeration from wind and waves
4) Photosynthesis of aquatic plants

There are a number of factors that affect the DO concentration including:

1. Efficiency of re-aeration from the atmosphere: Oxygen is easily transported from air to water in shallow, turbulent streams. It is poorly transported in deep, slow-moving or stagnant streams.

2. Organic materials in water material such as food processing wastes, human and animal feces and urine, paper mill wastes, dead and decomposing algae and leaves, etc. can affect the levels of DO in water. These materials when present in water are referred to as Biochemical (or Biological) Oxygen Demand (BOD) and can be used as food by bacteria naturally present in surface waters. As the bacteria feed upon these materials, they use oxygen. They also multiply. If there is sufficient BOD present, its metabolism by the stream bacteria will use up all of the dissolved oxygen in the water. At this point fish and most benthic macroinvertebrates die of suffocation.

3. Temperature: The solubility of oxygen in water decreases with increasing temperature. In other words, colder water is capable of holding more dissolved oxygen than warmer water. For example, at 14^{o}C the solubility of oxygen in pure water (no dissolved salts) is 10.30 mg/L, while at 30^{o}C it is only 7.56 mg/L.

There are a number of human-caused conditions that can cause stream temperature to rise. Some strategies for remediation for each of these are listed.

1) Runoff: Impervious surfaces, cultivated fields and lawns cause water to run into lakes and streams quickly. When this happens, the water is much warmer than if it soaks into the ground and slowly moves as groundwater to enter streams and lakes. Buffer zones, retentions ponds, rain barrels and water gardens can be helpful. Greater use of permeable asphalt that allows water to penetrate through to the soil would also reduce runoff.

2) Discharged water: Water that is discharged from industrial operations or water treatment plants is generally warmer than the bodies of water it is discharged into. Here, too, retention ponds for cooling could be helpful.

3) Health of Riparian Zones: Water that is shaded is cooler than water that is exposed to sunlight. When trees and shrubs that shade streams and rivers are removed, the increase in sunlight warms the water. Restoring streamside vegetation with a mix of trees, shrubs, grass and other plants will create more shade and also reduce erosion of stream banks.

4) Erosion: When soil enters the water through erosion, the increased concentration of sediment (dirt in the water) increases turbidity. Water heats up more quickly when it has high turbidity. Good vegetation buffer zones to prevent soil from entering streams will improve stream health in many ways.

For this and all other units, advanced level information is available if desired. Contact the HRWC and request an electronic version of the unabridged manual.

Procedure for Determining Amount of Dissolved Oxygen in a Water Sample

1. Fill stoppered bottle with the sample of water. Be sure no air is trapped inside.
2. Remove stopper and add DO 1 reagent. (DO= dissolved oxyen) Also add DO 2 reagent
3. Replace stopper without trapping air and, with a thumb on top of the stopper to hold it securely in place, turn the bottle upside down and back several times so that the chemicals mix and dissolve
4. Set the bottle down and watch while a precipitate (floc) settles. Let it settle until the bottle is about ½ filled with the precipitate
5. Again holding the stopper securely, turn the bottle upside down and back a few times and then let the precipitate settle once more until the bottle is half filled
6. Remove the stopper and add DO 3 reagent. The water will turn yellow-brown if O_2 is present
7. Fill the measuring tube to the top with the liquid and then pour it into the square bottle
8. Add sodium thiosulfate one drop at a time until the water is clear
9. Count the drops of sodium thiosulfate as they are added. Each drop =1mg of O_2 per liter of water

Dissolved Oxygen (DO) Data Sheet

<u>First run:</u> Drops of thiosulfate solution added to decolorize **one** measuring tube of sample: _____DO concentration = ______ mg/L Drops of thiosulfate solution added to decolorize **two** measuring tubes of sample: (DO concentration = half # drops added) _____DO concentration = ______ mg/L **<u>Second run</u>:** Drops of thiosulfate solution added to decolorize **one** measuring tube of sample: _____DO concentration = ______ mg/L Drops of thiosulfate solution added to decolorize **two** measuring tubes of sample: (DO concentration = half # drops added _____DO concentration = _____ mg/L	Minimum DO requirements for some aquatic organisms Trout 6.5 mg/L Smallmouth bass 6.5 mg/L Caddisfly larvae 4.0 mg/L Mayfly larvae 4.0 mg/L Catfish 2.5 mg/L Carp 2.0 mg/L Mosquito larvae 1.0 mg/L

1. Does this water meet the Michigan state requirement of 5.0 mg/L as the minimum acceptable DO concentration?

2. Could trout and smallmouth bass live in this stream?

3. Name 3 factors that affect Dissolved Oxygen concentration.

4. What can people do to improve the DO concentration in our watershed?

5. KEY Dissolved Oxygen Student Page

1. Does this water meet the Michigan state requirement of 5.0 mg/L as the minimum acceptable DO concentration?
   Answers will vary
2. Could trout and smallmouth bass live in this stream?
   Answers will vary
3. Name 3 factors that affect Dissolved Oxygen concentration

   a. Efficiency of re-aeration from the atmosphere. Efficiency of oxygen transport from air to water is high in shallow, turbulent streams; it is poor in deep, slow-moving or stagnant streams.

   b. Temperature. The solubility of oxygen in water decreases with increasing temperature. For example, at 14°C the solubility of oxygen in pure water (no dissolved salts) is 10.30 mg/L, while at 30°C it is only 7.56 mg/L.

   c. Presence of Biochemical (Biological) Oxygen Demand, BOD. BOD consists of organic material (food processing wastes, human and animal feces and urine, paper mill wastes, dead and decomposing algae and leaves, etc.) that can be used as food by bacteria naturally present in surface waters. As the bacteria feed upon the BOD, they use oxygen. They also multiply. If there is sufficient BOD present, its metabolism by the stream bacteria will use up all of the dissolved oxygen in the water. At this point fish and most benthic macro-invertebrates die of suffocation.

4. What can people do to improve the DO concentration in our watershed?
   a. **Help maintain natural streamsides by limiting erosion, leaving rocks in river bottoms. Remove un-needed dams.**
   b. **Limit erosion, since sediments can increase stream temperatures.**
   c. **Clean up after pets, limit discharge of biological wastes into streams.**

Dissolved Oxygen Lesson Narrative

Introduction, (5 minutes.) The mission is to make these points:
The presence of dissolved oxygen, O_2, in water, H_2O, is essential for the survival of fish and other aquatic critters.
DO in water is consumed by the respiration of aquatic animals, the stream bacteria that cause decay of dead vegetation and other carbon-containing material (sewage, food-processing waste, for example.

Transfer of O_2 from the atmosphere to water is a slow, inefficient process. It is faster when the water is shallow and turbulent (riffles, rapids, windy conditions)
Michigan's standard for DO is 5 mg/L, below which fish and other aquatic life may suffer or die.

1. My name is ________, and I'm a volunteer with the Dissolved Oxygen station. Please tell me your names. (Go around.)
2. So what do you know about how aquatic critters breathe? (Solicit 2-3 answers.)
3. The presence of dissolved oxygen, O_2, in water, H_2O, is essential for the survival of fish and other aquatic critters. They have to breathe, just as we do, but generally use gills or their skin for this, rather than lungs.
4. The concentration of oxygen in the air we breathe is about 270 mg/L. Let's make some guesses about how much dissolved oxygen, DO, there is in water like this stream. (2-3 guesses)
5. Actually, DO concentrations in water are quite low. The concentration of dissolved oxygen, DO, in water under the best conditions, about 9-10 milligrams per liter (mg/L.) This doesn't give aquatic life a very big safety margin.
6. So what are some of the things that can use up DO in water? (2-3 answers)
7. DO in water is consumed by the respiration of aquatic animals like fish and aquatic insects.
   Stream bacteria cause decay of dead vegetation and other carbon-containing material (sewage, paper mill waste, waste from livestock feeding operations, food-processing waste, for example).
8. How does oxygen get into water from the air? (2-3 answers)
9. To get from air to water, oxygen must be transferred across the air-water surface. Unfortunately, the transfer of O_2 from the atmosphere to water is a slow, inefficient process. It is faster when the water is shallow and turbulent (riffles, rapids, windy conditions. The more air-water surface the better.
   If the DO in the water is being used up faster than it's being replenished from the air, DO concentrations can approach zero, and we have trouble, like a big fish kill.
10. Michigan's standard for DO is 5 mg/L, below which many fish and other aquatic life may suffer or die.
11. Oxygen is less soluble in warmer water, and DO is used up most rapidly by bacterial decay processes when the water is warm, so problems with low DO in streams are most troublesome during the late summer and early fall.
12. Here we shall measure the concentration of DO in this stream using a chemical test kit that uses the Winkler method to see if the stream contains enough DO to support life. Note that this test only tests the water at this moment, and doesn't tell us about the water yesterday, last week or last year.

The Activity: 15 minutes

13. If there is poison ivy at the collection site, show all the students where it is and what it looks like.

Student 1 collects the water sample for DO. Wade into the stream, rinse the bottle in creek water, turn it upside down and submerge it well below the stream surface—close to the bottom in a shallow stream. Turn it right side up and let it fill, hold it in that position for a minute, then bring it to the surface, tip it slightly, and stopper it. Make sure no air bubbles are trapped in the bottle.

14. Put on your safety goggles. Open the DO1 reagent envelope and the DO2 reagent envelope. Remove the bottle stopper.
    Student 2 puts on safety goggles and adds the contents of a DO 1 reagent envelope. Student 3 puts on safety goggles and adds DO reagent 2 envelope . Stopper the bottle carefully to avoid trapping any bubbles.
15. Invert the bottle several times to dissolve the powders. A fluffy precipitate (floc) will form, brown if oxygen is present. Then let the floc settle to about half the bottle volume.
16. Invert the bottle again to mix, and let the floc settle to about half the bottle volume.
17. Remove the stopper. Open DO 3 reagent powder pillow .
    Student 4 puts on safety goggles, opens and adds one DO 3 reagent powder pillow. Invert the bottle several times to mix. Sample will turn yellow-brown if oxygen was present.
19. Have Student 5 add one full measuring tube of sample to the square bottle in the kit.
20. Have Student 6 add sodium thiosulfate solution dropwise to the square bottle (don't run the drops down the side of the bottle, and hold the dropper at an angle of about 45 degrees.)
    Have Student 7 swirl the bottle after the addition of each drop and count the number of drops added. Stop when the color of the solution changes from faint yellow to completely colorless. (This is easiest to see if done on a white background like a piece of paper.)
21. Student 8 counts records the number of drops of thiosulfate solution added; this is equal to the DO concentration in the water in mg/L.
22. Select new volunteers and repeat Steps 18-21 if time permits.
23. Average the results if you had time for more than one titration.

Dissolved oxygen concentration = ___________mg/L

The Wrap Up: 5 minutes

24. Ask the students if the stream meets Michigan's DO standard of 5 mg/L. Could trout thrive in it? (DO >= 6 mg/L)
25. Ask the students what aspects of the stream and the season are affecting its DO concentration. Are your DO results roughly what you'd expect? (depth, turbulence, season, possible pollution)
26. If you dump grass clippings or yard litter into a stream, how do you think this would affect the DO concentration in the stream? (Reduces DO, as this stuff decays it uses up oxygen.)

27. Do you expect low DO concentrations in a stream to be a problem in winter? (No; cold water holds more oxygen than warmer water, and consumption of DO by decay processes in the stream is slower when it's cold.
28. Thanks for stopping by and visiting with me today. It's time to move on to your next station.

Technical Postscript

If you have students who have taken or are taking high school chemistry, may ask you for the chemical equations involved in (1) the decay process, and/or (2) the Winkler method for DO.

We represent decay, which is actually an extremely complicated, multi-step process, by the metabolism of sugar (sucrose) to carbon dioxide and water:

: $C_{12}H_{22}O_{11} + 12\ O_2 \text{ ---> } 12\ CO_2 + 11\ H_2O$

342 g   12 x 32

= 384 g

Since 342 g of sucrose requires 384 g of oxygen, we see that 1.00 g of sucrose requires 1.12 g of oxygen. So if a wastewater contains only 50 mg of sucrose per liter, this will use up 56 mg of oxygen per liter of water as the bacteria feed upon it. We say that the BOD of this water is 56 mg/L. If the water is at 20^{o}C, it can contain a maximum of 9.09 mg/L of dissolved oxygen, not nearly enough to degrade all the sucrose. So, unless we provide additional oxygen from the air (by bubbling or shaking), this water will become anaerobic and deadly to aquatic life.

The equations for the Winkler method are as follows:

(1) Remove interfering NO_2^- (nitrite) from the water sample with alkaline NaN_3 (sodium azide);

$$HN_3 + NO_2^- \rightarrow N_2 + N_2O + OH^-$$

(2) Add $MnSO_4$ (manganous sulfate) and KI (potassium iodide) to the alkaline water sample; the reaction is

$$Mn^{+2} + 2OH^- \rightarrow Mn(OH)_2 \text{ (white ppt.)}$$

(3) The $Mn(OH)_2$ (manganous hydroxide) reacts with dissolved O2 to form brown MnO_2 (manganese dioxide),

$$Mn(OH)_2 + \tfrac{1}{2}\ O_2 \rightarrow MnO_2 \text{ (brown ppt.)} + H_2O$$

(4) Acidify the sample with H_2SO_4 (sulfuric acid) or HNH_2SO_3 (sulfamic acid); this results in the reaction

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

(5) Titrate the released I_2 (iodine, yellow-brown) with standardized solution of $Na_2S_2O_3$ (sodium thiosulfate); the volume of $Na_2S_2O_3$ solution required to cause the yellow sample solution to become clear is proportional to the concentration of D.O. in the original sample.

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