

Wastewater Treatment

Abstract: This activity investigates the process of aerobic metabolism (biodegradation) of organic material. Such metabolism by microorganisms is the basis for biological secondary wastewater treatment. This activity demonstrates the basis of all biological treatment processes, including trickling filters, activated sludge, oxidation ditches, and lagoons. This demonstration is an approximation of the biochemical oxygen demand (BOD) test typically run by wastewater plant operators to estimate the amount of biodegradable organic material in a sample.

Objectives: Be able to:

1. Explain the goals and the process of aerobic biological treatment.
2. List and explain the factors that affect the rate of aerobic biological treatment.
3. Explain how the BOD test is run and how it is used by wastewater plant operators.
4. Run the dissolved oxygen (DO) test.
5. Predict the results of changing the conditions of the BOD test.

State Standards Addressed:

Math - A2-4, A6, C1, D1-5, E1-3

Science - A14A-C, B1-6, D1-6

Time Frame & Schedule:

Preparation: 45 min.

Class: One 50 period for setup

15 min. each day during the next four days

One period for the final day

Materials: (K -Included in kit / O - Order / L - Obtain locally)

- ✓ Large container for fresh water sample (L)
- ✓ Six “BOD” bottles per group; 200 - 500 mL with cap. “Snapple” soft drink bottles or the equivalent work nicely. (L)
- ✓ “Karo” light corn syrup (L)
- ✓ “Schultz Instant Fertilizer,” NPK of 10-15-10 (L) (K)
- ✓ Pipettes (L)
- ✓ Large container to mix sample (L)
- ✓ DO Kit (Hach or LaMotte) (O) (K)

Teacher’s Preparation:

1. It is best to start this exercise on a Monday so that the test can be monitored daily for the first four days and then again on the following Monday.
2. Collect a freshwater sample that would be expected to contain both some organic material and a population of bacteria. Water that is turbid or colored will probably have adequate organics and bacteria. We have found, however, that some freshwater

- samples (lakes & streams) have adequate bacteria, but minimal organic material. For that reason it may be necessary to supplement the organics.
3. It might be advisable to do a trial run on your water sample using the recommended nutrient supplements. If a straight, un-supplemented, sample shows good DO depletion, you're okay. If DO is depleted too quickly, use less stock Karo in the BOD mixture. If DO does not change fast enough, use more stock Karo in the BOD mixture. (See Step #c below in *Explore*).
 4. Prepare Stock Solutions for nutrient supplement:
 - a. Liquid Plant Fertilizer Stock: (provides inorganic nutrients)
 - i) Dilute 1:200 with non-chlorinated water. Store in a capped brown bottle.
 - b. Karo Stock: (provides organic nutrients)
 - i) Dilute corn syrup 1:200 by adding 1 teaspoon of syrup to 995 mL of non-chlorinated This Karo stock is about 5 mg of sugar (glucose & fructose) per mL.
 - ii) Do not use table sugar (sucrose).
 5. Copy Fact Sheet and Data Sheet.
 6. Collect equipment, supplies, and other materials as required.

Description of Student Activities:

1. *Gear-Up:* (Work as teams of 3 or 4 students)
 - a. Show photos or overheads of various biological treatment processes.
 - b. Hand out Fact Sheet.
 - c. Lecture on the concepts of biological treatment and the BOD test.
 - d. Hand out Student Exercise Directions and Data Sheet.
 - e. Explain the *Explore* activity. The objective is to demonstrate that over a period of days the dissolved oxygen concentration in the bottles will decrease because the bacteria are metabolizing the organics and, in so doing, using up the oxygen.
 - f. Have the students develop a hypothesis about what they expect will happen to the DO during the test period.
2. *Explore:*

First Period:

 - a. Start with a set of six bottles that are all of the same volume and have a cap that closes the bottle without trapping any air under the cap. The usual BOD bottle is exactly 300 mL. However, any bottle from 200 mL to 500 mL will work for the exercise.
 - b. Label the bottles: #0 is the "initial DO" dissolved oxygen; #1 is "1-Day DO;" #2 is "2-Day DO;" #3 is "3-Day DO;" #4 is "4-Day DO;" and #7 is "7-Day DO."
 - c. Make up the BOD mixture:
 - i) Explain to the students why they are adding the supplemental nutrients and how you made up the stock. Help them calculate the total volume of mixed sample they will need for their six bottles.
 - ii) The total volume of mixed sample depends on the size of the bottles you are using. Multiply the volume of a single bottle by six and round up to the nearest liter to estimate the required volume of mixed sample. Overestimate slightly because bottles must be filled to overflowing.

- iii) Measure out the estimated volume of the freshwater sample to be tested and put it in an appropriately large flask or beaker.
 - iv) Add 1.0 mL of stock liquid fertilizer per liter of water sample and mix well.
 - v) Add 1.0 mL of stock Karo syrup per liter of water sample and mix well.
 - vi) Fill each of the bottles to overflowing so that water rounds-up on the top.
 - vii) Cap the bottle so that no air is trapped inside, then invert the bottle to be sure there are no air bubbles.
- d. Set bottle #0 aside to run the initial DO (dissolved oxygen).
 - e. Place bottles #1 through #4 and #7 in a dark place at room temperature. The standard BOD test is incubated at 20° C, so room temperature is an approximation. A dark cupboard or under a cardboard box and away from windows are good incubation locations. If they are stored in the light photosynthesis may occur, adding oxygen to the bottle.
 - f. Students will measure the DO in these bottles each day, at about the same time of day, for the next four days and the last on the eighth day (seven days of incubation).
 - g. Run the DO test on bottle #0:
 - i) The DO on bottle #0 is measured to establish a starting or initial DO level for all bottles.
 - ii) Use the DO Kit to measure the DO following the Kit's directions.
 - iii) Record the results on the Data Sheet.

2nd Day through 5th Day and 8th Day:

- a. On day two, after 24 hrs., run DO on bottle #1 and record the results on the Data Sheet.
 - b. On day three, after 48 hrs., run DO on bottle #2 and record the results on the Data Sheet.
 - c. Continue this process through the 5th day and then finally on the 8th day. The standard BOD is usually run for 5 days, but that is difficult to do in a five-day classroom situation.
3. *Generalize:*
- a. Review the data and discuss the results and questions. Did the students prove their hypotheses?
 - b. Have the students graph the data. Put time, in days, on the X-axis and DO consumed, in mg/L, on the Y-axis. DO consumed (at any time) is the initial DO minus the measured DO (at that same time).
4. *Apply:*
- a. Present the following questions as scenarios to analyze. Have the students discuss the questions among themselves and then have each group report to the class their answer and explanation to one of the questions:
 - i) "If you have a biological treatment plant and the amount of organic material in the influent increases, what would happen to the amount of oxygen consumed?" *It would increase.*
 - ii) "If you have a biological treatment plant and a lot of rainwater (runoff) gets into the influent, what would happen to the amount of oxygen consumed?" *It would probably decrease because the organics are diluted.*

- iii) “If you have a biological treatment plant and some cleaning solution (solvents) get into the influent, what would happen to the amount of oxygen consumed?”
It would decrease because the cleaning solution would be toxic to the bacteria and so their metabolism would decrease.
- iv) “If your treatment plant discharges its effluent into a stream and, because the plant is not doing a very good job, the effluent contains quite a lot of organic material, what would happen to the DO level in the stream? *It would be depleted because the natural bacteria in the stream would consume oxygen as they metabolized the organics. This would happen unless there was enough natural aeration to replenish the oxygen.*

Extension Activities:

1. The BOD test is used to estimate the amount of organic material entering a treatment plant (influent) and the amount of organic material in the plant effluent. From these data the operator can determine the percent removal of organic material accomplished by the treatment; in other words, how good a job he/she is doing. Ask the community operator to discuss with the class the plant influent and effluent BODs and how the plant is performing.
2. If the community has a biological wastewater treatment plant, ask the operator to describe the process and how he/she controls the growth of the bacteria.
3. Have the students select one type of biological treatment process and write a report on how microorganisms are used in that specific process to reduce the organic material in the wastewater.

Assessment:

1. Evaluate the questions on the Student Exercise Directions.
2. Evaluate report on the biological treatment process chosen in Extension Activities 3 above.
3. Evaluate the construction of the BOD versus time graph constructed by each student.
4. Evaluate oral report on analysis questions in 4a.above.
5. Evaluate entries in journal or log regarding the definition of BOD and an explanation of the BOD test procedure.

References:

1. “Introduction to Alaska Small Wastewater Systems,” Arasmith, Skeet and Ploetz, Holly, ACR Publications, Albany, OR, 1995.
2. “Operation of Wastewater Treatment Plants, Vol.1,” 4th Ed., California State University at Sacramento, Sacramento, CA 1994.
3. “Wastewater Treatment - The Student Resource Guide,” Water Environment Federation, Alexandria, VA, 1987

Resources:

1. Dissolved Oxygen (DO) Kits
Hach Model:
Hach Company

PO Box 389
Loveland, CO 80539-0389
1-800-227-4224
FAX 970-669-2932
Cat. #1469-00
\$46.00

OR

2. LaMotte Model:
Carolina Biological Supply Co.
2700 York Rd
Burlington, NC 27215
1-800-334-5551
FAX 336-584-0381
Cat. #D8-65-286
\$23.50

STUDENT EXERCISE DIRECTIONS

Wastewater Treatment

Purpose: This activity investigates the process of aerobic metabolism (biodegradation) of organic material. This demonstration is similar to the biochemical oxygen demand (BOD) test run by wastewater operators to estimate the amount of biodegradable organic material in a sample. A series of bottles is set up with organic material (food), bacteria and plenty of oxygen. The starting dissolved oxygen (DO) concentration is measured and the bottles are allowed to stand during which time metabolism occurs. Each day a bottle is opened and the DO measured. The change (depletion) in DO shows the amount of metabolism that has occurred since the beginning, due to the amount of organic “food” being consumed by the bacteria.

Activities:

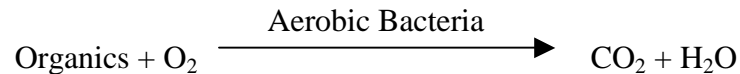
1. Obtain a copy of the Student Exercise Directions and Data Sheet for each group member.
2. Start with a set of six bottles that are all of the same volume and have a cap that closes the bottle without trapping any air.
3. Label the bottles: #0 is the “initial DO” dissolved oxygen; #1 is “1-Day DO;” #2 is “2-Day DO;” #3 is “3-Day DO;” #4 is “4-Day DO;” and #7 is 7-Day DO.”
4. Make up the BOD sample mixture:
 - a. The total volume of mixed sample depends on the size of the bottles you are using. Multiply the volume of a single bottle by six and round up to the nearest liter to estimate the volume of mixed sample needed. Overestimate slightly because the bottles must be filled to overflowing.
 - b. Measure out the estimated volume of the sample and put it in an appropriately large flask or beaker.
 - c. Add 1.0 mL of stock liquid fertilizer per liter of water sample and mix well.
 - d. Add 1.0 mL of stock Karo syrup per liter of water sample and mix well.
 - e. Fill each of the bottles to overflowing so that water rounds-up on the top.
 - f. Cap the bottle so that no air is trapped inside the bottle, then invert to be sure there are no air bubbles.
5. Set bottle #0 aside to be used for the initial DO reading.
6. Place bottles #1 through #5 and #7 in a dark place at room temperature. The standard BOD test is incubated at 20° C, so room temperature is an approximation. A dark cupboard or under a cardboard box and away from windows are good incubation locations. If they are stored in the light photosynthesis may occur, adding oxygen to the bottle.
7. Measure the DO in these bottles each day, at about the same time of day, for the next four days and the last on the eighth day.
8. Run the DO test on bottle #0:
 - a. The DO on bottle #0 is measured to establish a starting DO level for all bottles.
 - b. Use the DO Kit to measure the DO, following the Kit’s directions.
 - c. Record the results on the Data Sheet.

Fact Sheet

Wastewater Treatment

The secondary treatment phase of a typical wastewater treatment plant will usually be a biological treatment process. Biological treatment is so-called because the process uses biological organisms (primarily bacteria) to digest the organic material in the wastewater. Biological treatment processes include the activated sludge process, trickling filters, oxidation ditches, and lagoons. These processes are metabolically similar; they all utilize aerobic (in the presence of oxygen) metabolism by the bacteria.

In biological treatment processes, microorganisms metabolize or breakdown organic constituents (pollutants) in household waste to carbon dioxide and water. The biochemical reaction is summarized as follows:



This is called aerobic metabolism because oxygen is required for the reaction to occur. Oxygen in the above reaction is supplied by aeration in activated sludge treatment plants or trickling filters and by photosynthesis and surface wind in ponds and lagoons.

Biological organisms that provide treatment may be either suspended (activated sludge, ponds, lagoons) or attached (trickling filters and rotating biological contactors). These microorganisms use most of the suspended and dissolved material found in the wastewater as their food. They are chiefly aerobic using oxygen as they eat and grow, just as humans and other animals do.

The biological treatment process environment is designed to provide controlled conditions so that the microorganisms can remove most of the organic material from the wastewater as it passes through the treatment facility.

The purpose of the biological treatment process is to decompose as much organic matter from the wastewater as possible. The process protects the receiving stream from organic loads that could lower dissolved oxygen enough to kill fish or otherwise be harmful. Plant effluent is considered "high quality" if it contains a very low level of organic material.

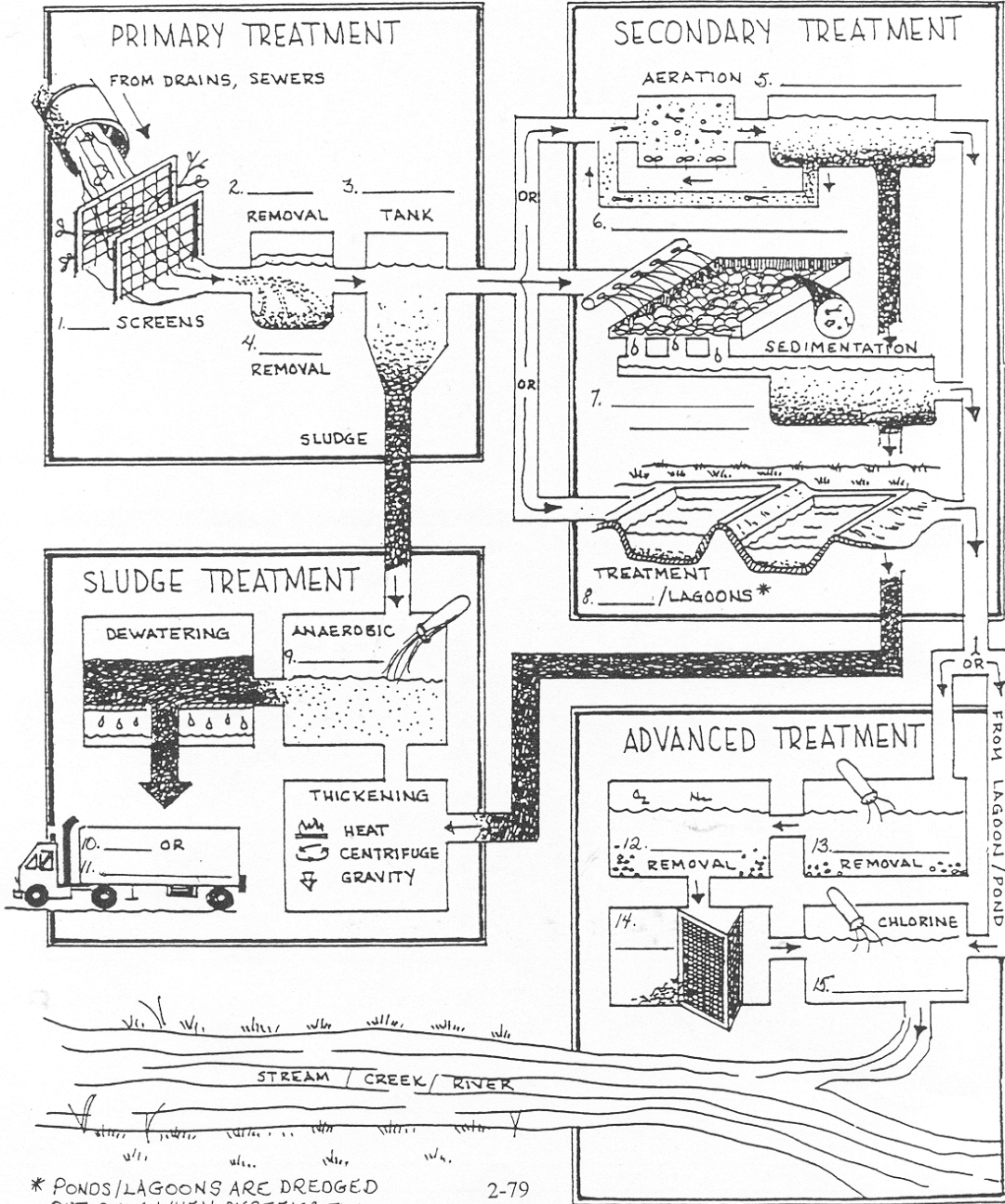
The biochemical oxygen demand (BOD) test gives a rough indication of the amount of biodegradable organic matter in a sample. The test also demonstrates the process of aerobic metabolism.

In the BOD test two bottles are filled with the wastewater to be tested. The wastewater contains organic matter, bacteria, and a good supply of dissolved oxygen (DO). The DO in one of the bottles is measured at the starting time. The second bottle is incubated at 20° C for 5 days in the dark. During those 5 days the bacteria metabolize the organic

matter and, to do so, use oxygen in proportion to the organic food. The DO in the second bottle is measured after the 5th day. The DO level should be lower because some was used by the bacteria.

A large DO depletion indicates a high BOD; high amounts of organics in the sample. Operators use the BOD test to estimate the amount of organics in the influent wastewater and in the plant effluent. The difference between these two BODs will tell the operator how much organic matter is being removed (metabolize) by the microorganisms in the biological treatment process.

TYPICAL WASTEWATER TREATMENT FACILITY



Data Sheet Wastewater Treatment

Name _____

Date _____

| Bottle # | Time, days | DO, mg/L | DO Consumed, mg/L |
|-----------------|-------------------|-----------------|--------------------------|
| #0 | 0 (initial) | | |
| #1 | 1 | | |
| #2 | 2 | | |
| #3 | 3 | | |
| #4 | 4 | | |
| #7 | 7 | | |

DO consumed in mg/L (at any time) = Initial DO in mg/L - DO in mg/L (at that same time).

General Safety Considerations

Only particularly unique safety issues are addressed in the individual units. However, teachers should instruct students in general laboratory safety practices and expect them to be followed at all times. Following are general safety considerations.

- Safety Equipment: Locate and know how to use all of the safety equipment in the lab. This includes fire extinguishers, fire blanket, safety shower, eyewash, Material Safety Data Sheets (MSDS), and first aid kit.
- Personal Protection: Wear goggles to protect your eyes whenever you are using chemicals or potentially pathogenic samples. Wear lab aprons to protect your clothes from chemicals. Wear rubber gloves when handling strong acids and bases and latex gloves when handling potentially pathogenic samples.
- In Case of Fire or Accident: In case of fire or accident, call your teacher at once. Report even minor incidents (such as small cuts, chemical spills, broken glassware, etc.).
- No food or drink in the lab: To avoid ingestion of harmful chemicals and potentially pathogenic samples do not bring food or drink into the laboratory area. Gummed labels should never be moistened with your tongue. Use tap water instead. Don't put your pencil or pen in your mouth.

Wash your hands thoroughly when you leave the lab and before you eat. This applies to short trip to the restroom as well as at the end of the period.

- Be cautious of hot plates and gas burners: To avoid burns, beware of hot plates and gas burners. Use protective gloves or long handled tongs when using autoclave, hot plate, furnace or oven. Never leave a burner unattended. Turn it off if you leave your lab table. Don't ignite it unless it is actually needed. If it is not working properly or does not light easily, ask your instructor for help. Long, loose sleeves and long hair are a fire hazard. Be careful. Pin hair back with a barrette or rubber band.
- Discard broken glassware appropriately: Discard or repair cracked or broken glassware immediately. Discard broken glassware in appropriate container, not the trash can.
- Pipeting: **NEVER** use your mouth to fill pipettes. Use a suction bulb every time.
- Consider all Chemicals as Dangerous: Always label chemicals properly. Never use chemicals or solutions from an unlabeled container. Never allow a chemical or solution to touch your skin. If you do, wash the area thoroughly with soap and water.
- Strong Acids and Bases: Be cautious of strong acids and bases. Always use

protective eyeglasses, gloves and apron when handling acids and bases. Always dilute strong acids and bases by adding the acid or base to the water, not *visa versa*.

- Samples: Most of the samples you work with will be harmless, but some are not. Wastewater samples and some freshwater samples may contain pathogens. To be safe, assume all of them to be potentially dangerous and handle them accordingly.
- Used Cultures: Place old cultures or any contaminated material in the biohazard bag provided by your teacher. Label all current culture tubes and dishes that you wish to keep with your initials, date and lab period (class).
- Spilled Cultures: Whenever bacterial cultures are accidentally spilled, notify your teacher so that proper disinfection procedures can be assured.