WATER QUALITY LAB

WATER QUALITY MONITORING
STUDENT HANDOUT

Part 1

PURPOSE

How can adding nutrients or heat to water change it as a habitat?
In this lab you will compare the productivity of a yeast culture at varying nutrient concentrations.

BACKGROUND

If partially treated sewage and other wastes are placed into water, they serve as food for microorganisms. The population of decomposers increases as the quantity of nutrients increases. The increase in microorganisms will change water in ways you will discover by doing this lab. When microorganisms consume organic matter in a water solution, they also use oxygen in the water, just as people use oxygen to metabolize their nutrition. The amount of oxygen needed for all of the living things in an ecosystem is called the Biological Oxygen Demand, or BOD. As the number of organisms in a system increases so does the BOD.

When we circulate water from a lake, river, or the ocean, through an industrial plant to help cool equipment, the water is placed back into its natural source at a higher temperature than when it left. Increasing water temperature also changes the water as a dwelling place for organisms. You will look at some environmental factors that are altered as the water temperature rises.

As the temperature of a liquid rises, its ability to hold a dissolved gas decreases. This is what happens to a "Coke." A warm "Coke" goes flat faster than an ice cold "Coke." An "Alka-seltzer" will "bubble" faster in a glass of hot water than in a glass of cold water. Similarly, cold water can hold more of the gas produced by the "Alka-seltzer" than warm water. Oxygen is a gas and more of this gas can be dissolved in cold water than warm water. Refer to graph on page 5.

Your test solution represents polluted water that is being "cleaned" by the action of a microorganism, yeast. In order to remove the pollutant (milk), the yeast consumes some of the dissolved oxygen (D.O.) from the solution. The amount of D.O. consumed depends on the pollutant concentration. The difference between the initial D.O. concentration and the D.O. minimum is a measure of the amount of D.O. needed to degrade the pollutant, and this value is referred to as the biological oxygen demand or BOD. The more organic matter in the solution, the more oxygen is demanded by microorganisms to remove it, and the greater the BOD.
Methylene blue is an indirect indicator for oxygen. During respiration, as the yeast consumes the available food, oxygen is also consumed and carbon dioxide is produced. The blue color disappears as the oxygen concentration drops and the carbon dioxide level rises. Carbon dioxide combines with water to form carbonic acid which changes the indicator color.

MATERIALS

clock (that indicates seconds)
600 mL beaker
3 100 mL beakers
six test tubes
test tube rack
thermometer
top loader balance (0.01 g)
10 mL pipettes
10 mL pipette pumps
hot plate
stirring rod
yeast
25 mL nonfat milk (per group)
.1% methylene blue solution
or bromthymol blue
25 mL graduated cylinder
Permanent Marking Pen

PROCEDURE

1. Obtain a 600 mL beaker and a thermometer. Fill the beaker half full of tap water and place the thermometer in it. Record the initial water temperature on the answer sheet (Question #1).

2. Now place your beaker of water used in the previous step on the hot plate and gently warm it to 40° C. It doesn't have to be exactly 40°C.

Caution: The surface of the hot plate will be much warmer than 40° C.

3. While the water is heating, obtain six test tubes, a test tube rack, three 100 mL beakers, and a 10 mL pipette & green pipette pump. Label the beakers: yeast, 100% milk, and 25% milk. Label the test tubes 1A, 1B, 2A, 2B, 3A, 3B with the Sharpie. (Note: Do NOT use a grease pencil.)

4. Place 1.0 g of yeast in the beaker labeled yeast and add 20 mL of tap water using the 25 mL graduated cylinder. Gently stir the mixture with a stirring rod, then rinse the stirring rod.

5. Pour 20 mL of nonfat milk into the beaker labeled 100% milk, Caution: When transferring a small quantity of a solution from one small container to another, both containers should be held. If not held, a slight jar could cause one of the containers to spill.

6. Pipette 5 mL of nonfat milk from the beaker in Step 5 into the beaker labeled 25% milk. Rinse the pipette. Using the graduated cylinder, add 15 mL of tap water. Stir with a glass rod. Rinse the glass rod after stirring.

7. Prepare the test tubes as indicated in the chart below, using a 10 mL pipette to measure the liquids. Record their color.

TABLE I. OXYGEN LOSS DATA TABLE

Test tube	H_2O	100% Milk	25% Milk	Methyl-ene blue	Color	Time *Yeast* Added	New Color	Time of Color Change	Elapsed Time
1A	3 mL	--	--	2 drops					
1B	3 mL	--	--	2 drops					
2A	--	--	3 mL	2 drops					
2B	--	--	3 mL	2 drops					
3A	--	3 mL	--	2 drops					
3B	--	3 mL	--	2 drops					

8. Place tubes #1A, #2A, and #3A in the 40°C water bath. Leave tubes #lB, #2B, and #3B in the rack.

9. Using clean equipment, pipette 2 mL of yeast solution into each of the tubes and stir each one. Record the time, to the second, that you add the yeast to each tube in the chart above.

Warning: Do not **shake** the test tubes once you have mixed in the yeast. (Why wouldn't you want to shake the test tube?)

10. Record the time it takes for each tube to turn from blue to white. A blue ring may remain at the top of the solution or a very light greenish-tan color may remain in the solution.

11. Tubes 1A and 1B should not change color. They are the control or comparison. What might cause a color change in the controls?

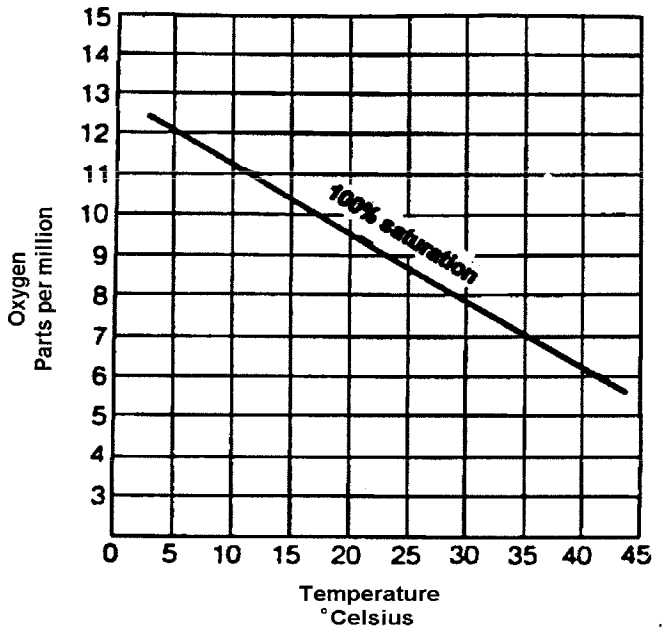
QUESTIONS

1. What is the initial temperature of the water? _____

2. What is the function of tubes 1A & 1B? _____

_____

3. This graph indicates the maximum concentration (saturation) of oxygen that water can hold at a particular temperature. On the graph's 100% saturation line, mark a dot representing the water temperature you recorded.



Dissolved Oxygen Saturation Point for Pure Water

4. The oxygen content of water is measured in parts per million (ppm). How many ppm of oxygen would the water in your sample

hold if it were saturated? _____

5. On the 100% saturation line make dots corresponding to 10°C and 40°C. Observe the ppm corresponding to 100% saturation at each of the three temperatures. At which temperature is the

concentration of oxygen highest? _____

6. What does the color change in tubes 2A, 2B & 3A, 3B imply about oxygen levels? _____

7. What does the change in color imply about the concentration of carbon dioxide and water? _____

8. What variable is changing between tubes 2 & 3 and how did that affect the elapsed time? _____

9. Each of these test tubes simulates a unique, small, environment. What is happening to the yeast populations?

10. How can it be harmful to supply an ecosystem with excess food (energy)?

11. How will this affect the available oxygen for the rest of the community? _____

12. How could methylene blue be used by industries to detect the presence of microorganisms in milk? _____

_____

_____

13. Without calculating the numbers, which test tube had the highest BOD? _____

14. How does a change in temperature affect the number of organisms that an environment can support? _____

_____

15. How is the change in temperature affecting the level of dissolved oxygen? _____

_____

16. What would expect to happen to the yeast population in the future? _____

_____

17. Power plants use water for cooling some of the machinery. If the water from a lake or river flows around the machinery in a power plant, even though the water never contacts any chemicals, in what sense could the water become polluted?

_____

_____

_____

Part II

PURPOSE

What is the oxygen content of a water sample? In this lab, you will measure oxygen content using a meter. The meter will give you readings for oxygen in parts per million, (ppm), for the solution being tested. You will graph your results.

BACKGROUND

From the introductory lab it can be seen that altering the temperature or nutrient content of water will change it as a habitat. During this lab you will measure oxygen content using a meter (quantitative). In such a meter, oxygen diffuses through a membrane into a system where the current conducted by oxygen ions can be measured. The meter will give you readings for oxygen in parts per million (ppm), for the solution being tested. You will graph your results.

MATERIALS

600 mL beaker
25 mL graduated cylinder
Yeast solution:
(2.0 g yeast/40 mL H_20)
glass stirring rod
balance top loader(0.01 g)*
100 mL of nonfat milk and
100 mL of water (50% soln.)
D.O. (dissolved oxygen) meter
100 mL beaker

PROCEDURE

Careful: The readings for your lab will be taken from the dissolved oxygen meter. It is important that the meter's probe be kept moist. Remember to return the probe to a beaker containing water so it doesn't dry.

1. Weigh-out 2.0 g of yeast on the balance. Add the yeast to 40 mL of tap water in a 100 mL beaker. Mix well with a stirring rod. Set aside.

2. Place 200 mL of 50% nonfat milk (100 mL milk + 100 mL water) into a 600 mL beaker. Read the temperature scale from the D.O. meter by turning the knob to temperature. Remove the probe from the water and place it in the nonfat milk solution. (Be sure that the probe does not touch the bottom or sides of the beaker, and is continually moved as you take your reading.) Record the initial temperature on the chart on page 9. Return the knob to the D.O. reading.

3. Immediately take the D.O., or dissolved oxygen, reading and record your answer on the chart.
4. Now add the yeast solution to the nonfat milk and swirl or mix with the probe (Be sure that the probe does not touch the bottom or sides of the beaker, and is continually moved as you take your reading.). Note the time in seconds when the yeast is added and record the D.O.

5. Continue to record D.O. at 15 second intervals until you get four consecutive readings that are the same or for a maximum of 10 minutes.

6. Record the ending temperature.

7. At the completion of your measurements carefully remove the probe and place it back into the water solution. Clean your lab station and equipment and return your equipment to its proper location.

Table II. Dissolved Oxygen Readings in Milk/Yeast Solution at 15 Second Intervals.

Beginning temperature_____ Ending temperature _____

Time	D.O.
Start	
0:00	
0:15	
0:30	
0:45	
1:00	
1:15	
1:30	
1:45	
2:00	
2:15	
2:30	
2:45	
3:00	

Time	D.O.
3:15	
3:30	
3:45	
4:00	
4:15	
4:30	
4:45	
5:00	
5:15	
5:30	
5:45	
6:00	
6:15	
6:30	

Time	D.O.
6:45	
7:00	
7:15	
7:30	
7:45	
8:00	
8:15	
8:30	
8:45	
9:00	
9:15	
9:30	
9:45	
10:00	

QUESTIONS

1. Graph your results.

2. Did you start with a saturated solution? (Use your first reading and the chart on page 5.) _____

3. At anytime, did you have a saturated solution? Why, or why not? _____

_____

4. As the yeast organisms consumed the milk, the D.O. concentration first fell rapidly and then more slowly until the D.O. no longer changed with time. This baseline value is the D.O. minimum. Determine the D.O. minimum for your solution.

_____

5. Explain what was happening in your yeast solution to cause the D.O. to drop. _____

6. Why does D.O. stop decreasing after a period of time?

_____

7. The amount of oxygen needed for all of the living things in an ecosystem is called the biological oxygen demand, or BOD. As the number of organisms in a system increases so does the BOD. The difference between the initial D.O. reading and the D.O. minimum reading is a measure of the amount of D.O. required for the milk to be consumed. This value is the BOD. Determine the BOD for the yeast. _____

8. Why do most people aerate their tropical fish tanks?

_____

9. Both the methylene blue and the D.O. meter tell you something about the oxygen content of a solution. Compare the two methods of measurement. What does each tell you? Why is each useful?

PART III

PURPOSE

How can we check for the presence of contaminating bacteria in water supplies? In this lab you will culture coliform bacteria found in water samples, count the colonies produced, and compare that data to permitted levels.

BACKGROUND

To insure that the water we use is acceptably free of contaminants, a test for coliform bacteria is commonly done. *Escherichia coli*, (E. coli), is a common inhabitant of the large intestines of humans and other vertebrates. If tests show that E. coli are present in amounts larger than trace quantities, then the water is being contaminated with fecal wastes.

MATERIALS (per station/group)

50 mL sample of water to be tested
(it may need to be diluted if you think that it has a high concentration of organisms or particulate matter)
1 Millipore filter petri dish (per group)
2 mL endobroth growth media (per group)
1 medium absorbing pad (per group)
1 Millipore grid filter paper (per group)
1 Millipore filtration unit (to be shared)
600 mL beaker of ethanol (per class)
10 mL pipette (shared for measuring endobroth)
10 mL pipette pump (shared for measuring endobroth)

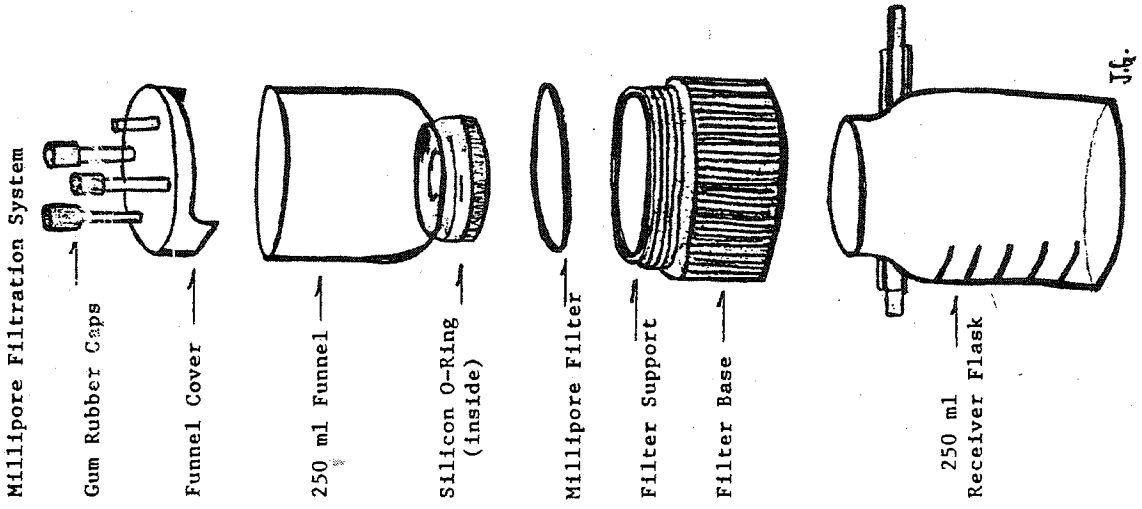


Figure 1. Millipore Filtration Apparatus

PROCEDURE

Day One

1. Assemble the Millipore Filtration Apparatus as shown in Fig. 1 on page 12.

2. Sterilize the tip of a pair of forceps in alcohol, then use the forceps to place one grid filter paper on the filter support, grid side up.

3. Gently screw the funnel to the filter support. Attach the funnel & filter to the receiving flask. Be sure the syringe pump mechanism is securely attached to the filter. Add 50 mL of water sample to be tested into the funnel (the funnel is graduated). Cap the funnel with the cover making sure three of the openings are covered with gum rubber caps and the fourth by the air filter assembly if needed.

4. Draw the water sample into the receiver by pumping the plunger until the water sample has been completely drawn through the grid printed filter.

5. Now prepare your petri dish by sterilizing the forceps again and using them to place a sterile white absorbent pad in the bottom half of the petri dish, the half that fits inside the other. Place your team's identification on the outside surface of this half of the petri dish.

6. Pipette 2 mL of the endobroth media onto the absorbent pad. (Caution: The stain in the media could be permanent in clothing.)

7. Carefully unscrew the funnel. Re-sterilize the forceps and use them to move the grid filter paper from the filter support base and place on the endobroth pad in the petri dish that you have just prepared. Be sure that the grid printed surface is on top. Immediately put the top back onto the dish and invert, so that your identification is on top. Incubate at 37°C for 24 hours or at room temperature for 48 hours.

8. Unscrew the receiver flask from the filter support and funnel assembly and pour the water down the sink. Return the apparatus so that the next team may start their filtering.

9. If instructed by your teacher, sterilize the filtration system in alcohol as follows: submerge the funnel and filter support into a beaker of 95% alcohol (ETHANOL). The receiver flask does not need to be dipped in alcohol. Wipe alcohol off with a paper towel.

10. Wash hands thoroughly with soap and water.

Day Two

11. When your petri dish is removed from the incubator or has set for 48 hours, view the

10. Wash hands thoroughly with soap and water.

Day Two

11. When your petri dish is removed from the incubator or has set for 48 hours, view the bacterial colonies. You may want to wipe the moisture from the top and reassemble the dish. If there are any coliform bacteria in your sample, they will appear as shiny colonies with metallic appearing surfaces. Non-coliform bacterial colonies will appear purple, without metallic appearing surfaces. If available, you may want to view your petri dish under a stereoscope.

12. Count the number of coliform and non-coliform colonies on your plate and record the information. Collect data for all water samples.

The acceptable amount of coliform bacteria in the water depends on where the water is and what the water is to be used for. The following table shows acceptable limits.

TABLE III. TOTAL COLIFORM PER 100 mL

Type of water	Desirable Level	Permissible Level
Drinking	0	4
Untreated water supply (reservoir)	50	200
Bathing and Swimming areas	200	1,000
Commercial boating	1,000	5,000

RESULTS

TABLE IV. COLIFORM/NON-COLIFORM SAMPLE COUNTS

SOURCE	COLIFORM	NON-COLIFORM	TOTAL BACTERIA	% COLIFORM	% NON-COLIFORM

QUESTIONS

1. Describe, not just name, the different water sample sources used by class members. Variables that you might consider in your descriptions: running, standing, closed, open, filter, treated,

use, oxygen content. _____

_____

_____

_____

_____

_____

_____

_____

_____

2. It is possible to test for all coliform, mammalian coliform, or for specifically human coliform. Which test would be the most

practical for recreational water? _____

_____

3. Which test would be the most reasonable for drinking water?

_____