DO NOT WRITE ON THIS

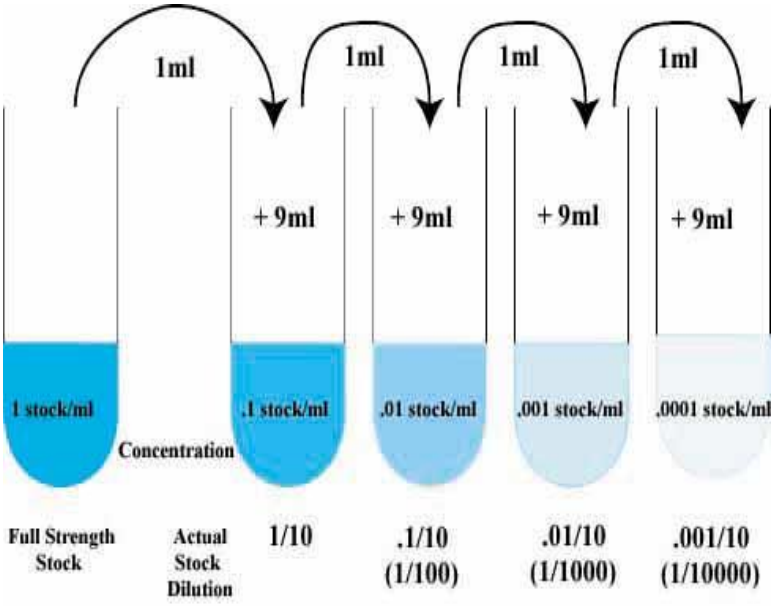
Dissolved Oxygen Lab

Biodegradable Materials and Their Effect on Dissolved Oxygen Levels

Purpose
In this laboratory exercise, you will design and conduct an experiment to evaluate the effect of the presence of biodegradable materials on dissolved oxygen levels.

Introduction
In aquatic systems, aerobic microorganisms will consume biodegradable material for energy, and in doing so will also take up oxygen from the environment as part of the cellular respiration process. Scientists use dissolved oxygen levels as an indication of contamination by such pollutants as sewage, agricultural runoff, organic industrial effluents, etc. *Biological Oxygen Demand* (BOD) is a measure of the oxygen used by microorganisms to decompose this waste. If there is a large quantity of organic waste in the water supply, there will also be a lot of bacteria present working to decompose this waste. In this case, the demand for oxygen will be high (due to all the bacteria) so the BOD level will be high. As the waste is consumed or dispersed through the water, BOD levels will begin to decline.

In this lab, you will be performing a serial dilution, which is a stepwise dilution of a substance in a solution. Through several steps, you remove a constant amount of liquid from a series of solutions and place it in a distilled water solution.



Methylene blue is clear in the absence of oxygen and blue in the presence of oxygen. The time for color change will vary depending on the concentration of biodegradable material.

Yeast is being used in this lab to represent the microorganisms. They are NOT the principle decomposers of biodegradable waste, but they are easily handled safely in a classroom setting. For this same reason, we are using milk as our biodegradable material because it is safer than the real-life alternative (sewage).

Pre-lab questions: *Answer the following questions on a separate piece paper. Use complete sentences.*

1. List 3 dissolved organic materials and possible sources that may decrease oxygen levels as a result of decomposition when present in water.
2. List 3 possible organisms that may act as decomposers of dissolved organic material in aquatic systems.
3. Develop a hypothesis for the effect of biodegradable materials on dissolved oxygen levels. What is your independent variable? Your dependent variable? Label and sketch a graph of the data you would expect based on your hypothesis.

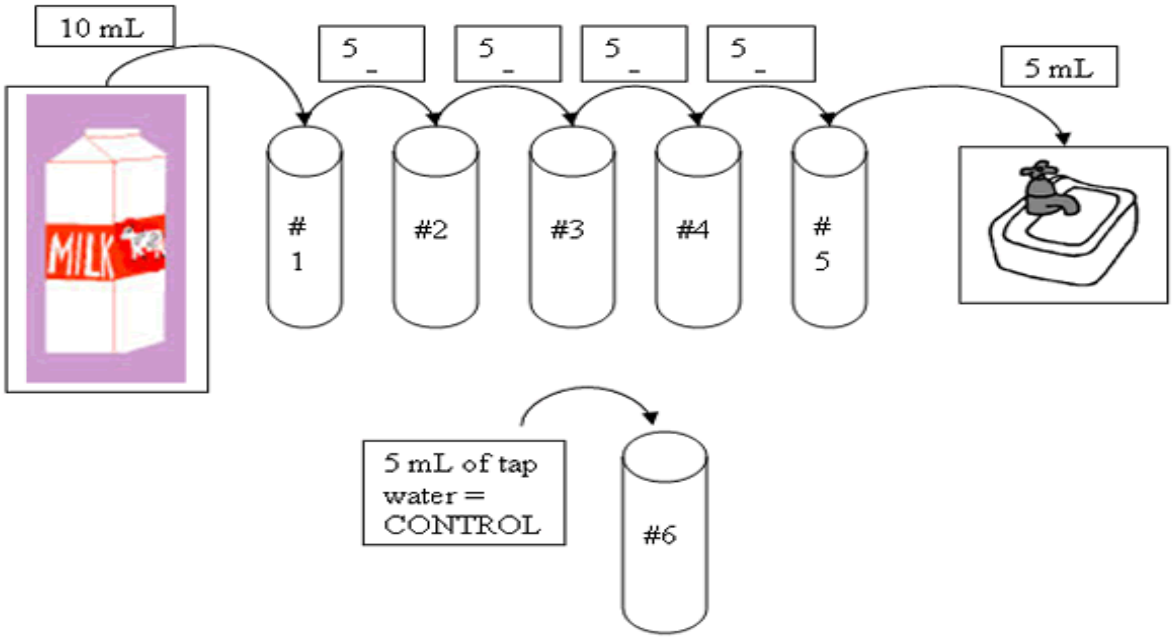
Materials

- Biodegradable liquid (milk)
- Six test tubes with screw cap or stopper - at least 10 ml capacity
- 10-ml graduated cylinder
- Test tube rack (see-through)
- Six 1 mL pipettes
- Yeast
- Methylene Blue (in dropper bottle)

Safety Measures
Methylene Blue may be harmful if swallowed, inhaled, and/or in contact with skin. It may cause severe eye irritation.

Procedure

1. Label the test tubes #1 through #6. Place them in the test tube rack.
2. Using a graduated cylinder, add 5 mL of tap water to tubes #2 through #6.
3. Add 10 mL of milk to tube #1.
   Steps 4-7 are illustrated in the diagram below. Refer to it as you read thru the steps.



4. Using a graduated cylinder, remove 5 mL of milk from tube #1 and place it in tube #2. Mix the contents well.
5. Remove 5 mL from tube #2 and place it in tube #3. Mix the contents well.
6. Remove 5 mL from tube #3 and place it in tube #4. Mix the contents well.
7. Remove 5 mL from tube #4 and place it in tube #5. Mix the contents well, and then discard 5 mL of the solution.
8. Add 3 drops of methylene blue (MB) to each tube. Be careful to hold the dropper bottle upright so that the drops are uniform. Mix each tube well.
9. Noting the time, quickly add 1 mL of yeast mixture to each test tube. Invert each test tube 4 times and place in the test tube rack.
10. Carefully observe each tube and record the time at which the color change from blue to white is complete (no more color change).The surface of each test tube will remain blue.
11. BEFORE you clean up, shake one of the test tubes that has turned white. Record your observations.

Data

1. Complete the table to show your serial dilutions.

Test Tube #	mL milk and/or H_2O	Methylene Blue	% Milk	Dilution
1	5	3 drops	100%	1:1
2	5	3 drops		
3	5	3 drops		
4	5	3 drops		
5	5	3 drops		
6	5	3 drops	0%	---

2. Record the time it takes for a color change.

Test Tube #	Time for the complete color change (blue to white)
1	11:26
2	21:00
3	20:30
4	11:04
5	12:42
6	16:40

3. Observations after shaking one of the test tubes?

Analysis and Conclusion

1. Graph your results. The x-axis should be your independent variable and the y-axis should be your dependent variable.
2. What does your graph tell you about the relationship between the biodegradable waste in water and the amount of dissolved oxygen in the water?
3. What is the gas taken in by the microorganisms?
4. What is the gas given off by the microorganisms?
5. Where do microorganisms living in water get the oxygen that they use in decomposition? (Hint: NOT from the oxygen in the water molecules).
6. Where do the green plants living in water get the carbon dioxide that they need?
7. What happened to the tube you shook after it had turned white? Why?
8. Which test tube had the most "sewage"?
9. What would be the effects of dumping a great deal of raw sewage into a river in regard to the dissolved oxygen in the same river?
10. Was your pre-lab hypothesis supported or refuted? Why or why not?