Oil Spill Bioremediation – Student Guide

Background

Each year, millions of gallons of oil enter the world's oceans. Dramatic accidents and oil spills – like the 2010 *Deepwater Horizon* disaster – make headline news, but incidents like these are to blame for only a small percentage of the oil polluting the world's oceans. The vast majority of ocean oil contamination originates from the accumulation of smaller, less publicized but commonplace events. The impact of oil pollution on marine ecosystems is profound and long-lasting. The oil that spilled during the *Deepwater Horizon* disaster, for example, decimated bird and fish populations and resulted in the deaths of dolphins, turtles, and deepwater corals; it also negatively impacted the commercial fisheries of the Gulf. Likewise, the harmful effects of the 1989 *Exxon Valdez* oil spill are still being felt in Alaska's Prince William Sound more than a quarter century after the tanker ran aground and spilled more than 10 million gallons of crude oil.

The toxic chemicals in floating oil can kill or contaminate plankton and algae. When fish eat these contaminated foods, they can die or become contaminated also. Fish larvae (or fry) can be killed, sickened, or disfigured, negatively impacting future population numbers. Those larvae that survive likely consume oil and/or oil dispersants. These compounds are transferred up the food chain when larger fish, birds, animals and humans ate these contaminated fish. This process is referred to as bioaccumulation. Heavy oil components sink to the ocean floor where they cover benthic (bottom-dwelling) organisms such as crabs, oysters, mussels, and clams. The toxicity of the oil either kills these organisms or penetrates their tissues, making them dangerous to consume. Oil also coats the feathers of birds and the fur of marine mammals, causing them to lose their natural insulation, buoyancy, and motility. Many of these animals drown; others die due to loss of body heat.

Some microorganisms living in the ocean have specialized metabolic pathways that enable them to use oil as food and convert it into energy. These microbes, mostly bacteria and some fungi, break down the long-chain hydrocarbons of petroleum and chemically convert them into energy and nutrients for their own biological processes. In doing so, they also transform the complex structure of oil into simpler, non-toxic by-products.

Scientists recognize great potential in utilizing the oil-degrading properties of microbes to expedite the breakdown of harmful oil from spills. The process of using living things to clean up environmental pollution is called bioremediation. Bioremediation using oil-degrading microbes causes minimal physical disruption to the environment, permanently removes harmful oil, is cost effective, and causes little damage to the ecosystem.

In this experiment, you will simulate bioremediation of a marine oil spill using microorganisms that consume oil. A chemical called tetrazolim is used as an indicator tor the breakdown of oil. Tetrazolium typically is colorless (when oxidized) but turns pink when its chemical composition is changed (when reduced). When microorganisms metabolize the carbon compounds in the oil, they create by-products that reduce tetrazolium and cause it to turn pink.

Pre-laboratory questions

1. The Deepwater Horizon incident resulted in the release of an estimated 4.9 million barrels of crude oil.
    a. One barrel of oil equals 42 U.S. gallons. About how many gallons of oil were released during this spill?

    _____

    _____

    _____

    _____

    _____

    b. Describe a method scientists may have used to estimate the amount of oil released in this spill. (**Hint:** How would you estimate the amount of water that would be lost from a faucet left running for 87 days?)

    _____

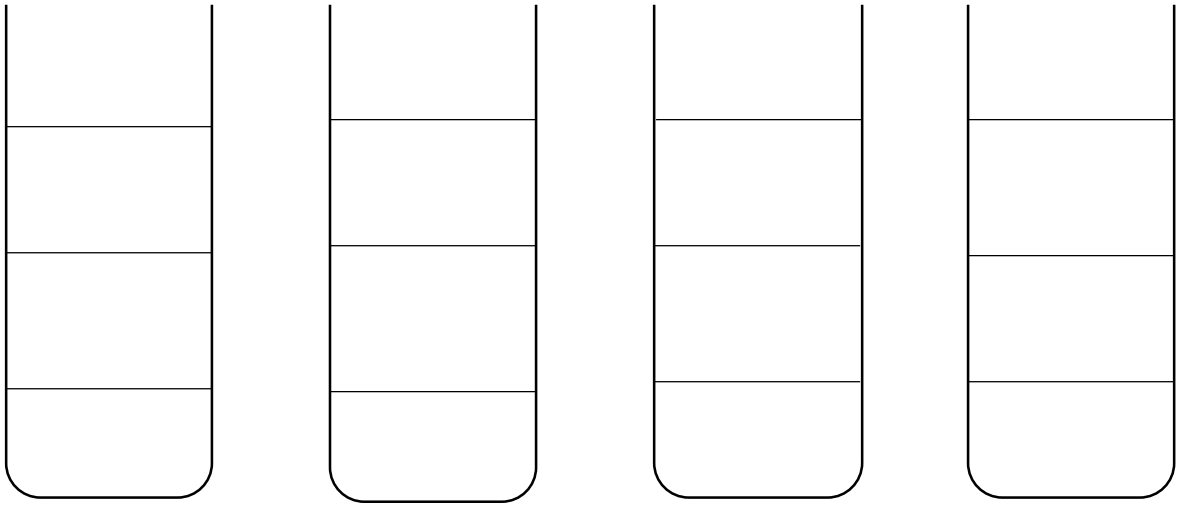
    _____

    _____

    _____

    _____

2. Read the procedure and then label the chemicals to be added to each. Refer to this drawing while performing the procedure.



Materials

5 pipets
4 culture
Pencil

Shared materials:

Test tube rack (or beaker for holding tubes)
Tetrazolium
Distilled water
Oil
Microbial suspension

Procedure

1. Use a pencil to label the culture tubes 1, 2, 3, and 4, consecutively, along with your name and the date. Tubes 1 and 2 will be used in Experiment A. Tubes 3 and 4 will be used in Experiment B.
2. Using a plastic pipet, add 2-mL of 0.02% tetrazolium indicator to tubes to tubes 1 and 2only. Use the graduations marked on the plastic pipet to measure the 2-mL amount. Discard the pipet when finished.
3. Using a clean plastic pipet, and 2-mL of distilled water to tubes 3 and 4 only. Use the graduations marked of the plastic pipet to measure the 2-mL amount. Discard the pipet when finished.
4. Using a clean pipet, add 10 drops of oil to all four tubes. Discard the pipet when finished.
5. Using a clean plastic pipet, add 2-mL of distilled water to tubes 1 and 3 only. Use the graduations marked on the plastic pipet to measure the 2-mL amount. Discard the pipet when finished. Cap tubes 1 and 3. (Use parafilm if you do not have test tube caps.)
6. Using a clean plastic pipet, add 2-mL of microbial suspension to tubes 2 and 4 only. Use the graduations marked on the plastic pipet to measure the 2-mL amount. Discard the pipet when finished. Cap tubes 2 and 4. (Use parafilm if you do not have test tube caps.)
7. Mix the liquid in all four tubes by finger vortexing them, one tube at a time. Hold the top of the tube securely in one hand; draw the index finger of the other had toward you several times, gently tapping the side of the tube near the bottom. This creates a whirlpool inside the tube, which mixes the liquid. Repeat this procedure with the remaining tubes. Place all four tubes upright in a test tube rack (or beaker is a rack is unavailable).
8. Record your initial and daily observations in the Lab Dara table on your data sheet. For experiment A, observe the color of the liquid in the tubes. For Experiment B, observe the appearance of the oil layer in the tubes. Record the color and the fluidity of the oil on each day. To aid in your observations, hold the tubes 3 and 4 up to the light in a horizontal position, and observe how the oil moves over the liquid. Invert tubes 3 and 4 several times and watch the oil gather back at the top pf the liquid. Observe any differences in the composition of the oil. Manipulate the tubes in any way that allows you to better view the characteristics of the oil in each. Finger vortex the tubes daily, as describes in Step 7.

Lab Data

	Experiment A observations		Experiment B Observations	
Day	**Tube 1**	**Tube 2**	**Tube 3**	**Tube 4**
0 (Initial setup)				
1				
2				
3				

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Questions

1. Describe how Experiment A was set up. What was the variable? What was the purpose of Tube 1?

2. Describe and interpret the result of Experiment A.

3. Describe how Experiment B was set up. What was the variable? What was the purpose of Tube 3?

4. Describe and interpret the result of Experiment B.