Oil Spill Bioremediation

Investigation Manual

CAROLINA DISTANCE LEARNING

OIL SPILL BIOREMEDIATION

Table of Contents

Overview

In this investigation, students will simulate the bioremediation of a marine oil spill. **Bioremediation** is the use of living things to clean up environmental pollution (in particular, microorganisms that consume oil). Students will apply a suspension of oil-degrading microbes to a small amount of oil and chemical indicator in a culture tube. A change in the color of the chemical indicator signifies a breakdown in the chemical structure of the oil.

Outcomes

- Describe the chemical nature of oil
- Explore the general process microbes use to break down oil

Time Requirements

Preparation ..... 30 minutes
Activity 1: Bioremediation of Oil ..... 45 minutes, then 10 minutes a day for 3 days

Key

Personal protective equipment (PPE)

goggles gloves apron

follow link to video

photograph results and submit

stopwatch required

warning

corrosion

flammable

toxic

environment

health hazard

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Background

Each year, millions of gallons of oil enter the world's oceans. The impact of oil pollution on marine ecosystems is profound and long-lasting. Dramatic accidents and oil spills—like the 2010 Deepwater Horizon disaster (also known as the BP oil disaster)—make headline news. 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 in the Gulf of Mexico. Likewise, the harmful effects of the 1989 Exxon Valdez oil spill are still 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.

However, these large incidents account for only about 5% of the oil polluting the seas that is a result of human activity. The vast majority of ocean oil contamination originates from the accumulation of smaller, less publicized but commonplace events, such as leaks from smaller oil tankers, routine operation of oceanic oil wells, leaking storage tanks and pipelines for offshore oil wells, and improperly drilled holes in the ocean floor. Loading and unloading tankers with oil for transfer from offshore rigs to onshore sites also can introduce oil into the ocean. Refined oil (i.e., fuel oil, gasoline, and other processed petroleum products) from municipal and industrial sources is often accidentally or deliberately dumped, spilled, or leaked on land

Figure 1.

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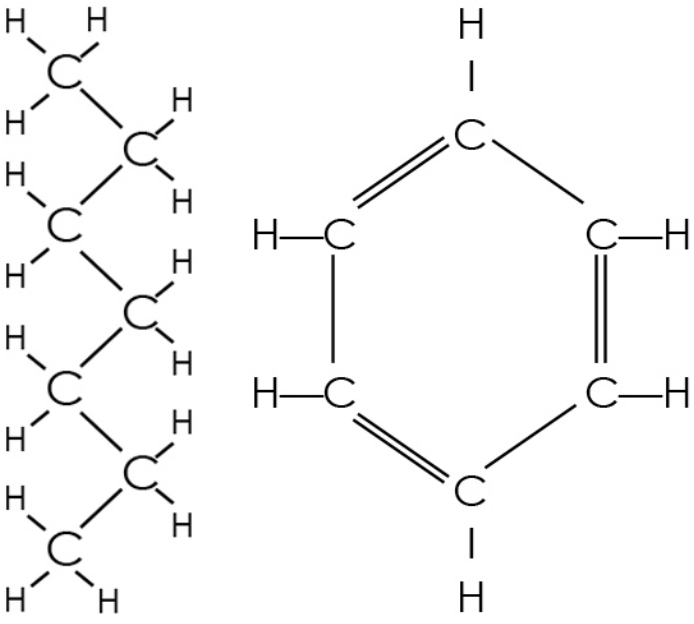
Background continued

and into waterways. Oil on roadways from motor vehicles is carried to waterways and eventually to the ocean by heavy rainfalls. Many people also dispose of used motor oil improperly. This oil can enter storm drains, streams, and rivers and can be carried out to sea. Oil can also enter the atmosphere as smoke from oil fires and then be deposited into the ocean and tributaries with precipitation.

Crude oil is a complex mixture of several types of hydrocarbons. A hydrocarbon is a chemical compound made entirely of carbon and hydrogen that usually forms a long chain, which can be either linear or in the form of a ring (see Figure 2). The toxic chemicals floating in oil can kill or contaminate plankton and algae. When fish eat these contaminated foods, they can also become contaminated or even die. Fish larvae can be killed, sickened, or disfigured, negatively impacting future population numbers. The larvae that survive likely continue to consume oil as well. These compounds are often transferred through an entire food web and can become more concentrated (have a higher potency) when larger fish, birds, other animals, and humans prey on 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.

Figure 2.



Heptane Chain **Benzene Ring**

Natural seepage of oil from oceanic oil deposits accounts for a significant amount of oil released into the ocean, but much of this natural seepage is consumed by ocean-dwelling bacteria that have evolved specialized pathways that enable them to use oil as food and convert it into energy. These microbes, containing 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, which is known as **biodegradation**. The hydrocarbons act as a carbon source from which the organisms build their biomass and grow. Many different species of oil-degrading microbes work together to break down the components of oil. These marine bacteria and fungi use enzymes

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and oxygen in seawater to break down the ring structures of the hydrocarbons, producing carbon dioxide (CO_2) in the process.

Scientists recognize great potential in utilizing oil-degrading microbes to expedite the breakdown of harmful oil from spills. There are three major approaches to this process, known as **bioremediation**, to clean up marine oil spills:

1. Manipulate the nutritional composition of the spill site to enhance the activity of indigenous oil-eating microbes.
2. Augment naturally occurring microbes with special mixtures of non-native, oil-degrading microorganisms.
3. Utilize genetically engineered microorganisms specifically designed to degrade oil effectively.

Optimizing the environment of oil-degrading microbes to accelerate their growth and reproduction is called **biostimulation**. Nutrient availability is the rate-limiting factor in microorganisms' ability to degrade petroleum in bioremediation. Nutrients such as nitrogen, phosphorous, and iron are necessary for indigenous oil-degrading microbes to convert the petroleum hydrocarbons into useful biomass and nontoxic by-products. These nutrients are typically in short supply because non-oil-degrading microorganisms compete to consume them. Through biostimulation, nutrients are added to the oceanic environment, much like applying fertilizer to a lawn. The amplified nutrient supply increases the rate and extent of microbial oil degradation. However, in order to encourage maximum microbe growth and oil breakdown, the nutrients must remain in contact with the oil and their concentration must remain at an optimal level for an extended time period. These conditions are difficult to maintain in dynamic aquatic systems.

A variety of chemical methods have been employed to disperse (break oil into smaller droplets) oil spills. The rationale for using dispersants is that, by breaking down the oil slick (area of oil floating on a body of water), the surface area of the oil is increased to allow more of the oil to be available for degradation by microbes. **Chemical dispersants**, also called surfactants, are classed by their ionic charge. Soaps are an example of an anionic (negatively charged) surfactant. The use of chemical dispersants is controversial, both because increasing the surface area of the oil also makes the toxins within the oil more available to the environment and because of the toxic nature of many of the surfactants.

The process of supplementing or "seeding" a population of naturally occurring, oil-degrading microbes with additional microorganisms is called **bioaugmentation**. This technique is often used when the existing population of microbes in a contaminated region is not optimally suited to degrade the type of oil present. Mixtures of microbial species that are better decomposers can be combined and grown in large batches in laboratories and then introduced at the spill site in bulk. Oil-degrading microbes can easily be cultivated in large quantities in laboratories and be ready for use when ocean oil pollution occurs. These microbial populations, called seed cultures, can be stored for up to three

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Background continued

years. However, recent studies following the cleanup effort in the wake of the Deepwater Horizon oil spill have questioned the efficacy of seeding microbe populations.

Breaking down petroleum is a complex biological process. A single species of an oil-degrading microbe cannot achieve complete degradation on its own. However, scientists are creating genetically engineered microorganisms (GEMs) in hopes to accomplish what natural species cannot. These GEMs are designed to incorporate the pathways and enzymes necessary to degrade oil more efficiently and thoroughly. The use of GEMs at spill sites has great potential, but it is a relatively new approach that is undergoing continued development.

In this investigation, you will simulate the bioremediation of a marine oil spill using microorganisms that consume oil. **Rid-X®** is a mixture of bacteria and enzymes that is used to maintain septic systems by degrading sewage, including oils. A chemical called **tetrazolium** is used as an indicator for the breakdown of oil. Tetrazolium typically is colorless (when oxidized) but turns pink when its chemical composition is changed (when reduced). When microorganisms break down the carbon compounds in oil, they create by-products that serve as electron donors (reducing agents). These electron donors change the chemical composition of the tetrazolium indicator (by the addition of hydrogen), causing it to turn pink (due to the creation of an insoluble pink compound). In this activity, the reduction of tetrazolium from its oxidized, colorless form to its reduced, pink form is used as an indication that the breakdown of oil is taking place.

The use of tetrazolium in this activity marks only the beginning of oil degradation. Complete decomposition of the oil would require abundant nutrients, involve several species of microbes, and occur over a long period, which cannot be completed in a scaled-down setting such as the one being carried out in this experiment. However, basic changes in oil composition can still be observed.

Materials

Included in the materials kit:

Rid-X® Septic System Treatment (powder), 20 g

10 plastic pipets, 3 mL, graduated

6 Culture tubes with caps

Bag containing tetrazolium indicator powder (0.02 g) and distilled water (100 mL), 0.02%

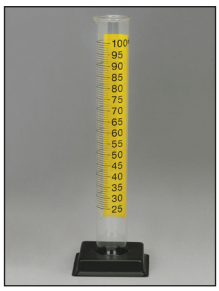
Cheesecloth

Funnel

Reorder Information: Replacement supplies for the Oil Spill Bioremediation investigation can be ordered from Carolina Biological Supply Company, item number 580830.

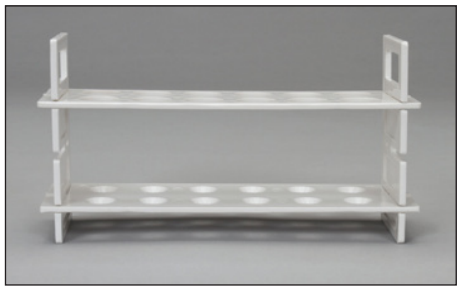
Call: 800.334.5551 to order.

Needed from the equipment kit:



Graduated cylinder

2 Plastic cups



Test tube rack

Needed but not supplied:

- Cooking oil
- Bottled water, 30 mL
- Warm tap water, 140 mL
- 10% bleach solution for cleanup
- Pencil
- Stopwatch (or cell phone with a timer)
- Camera (or cell phone capable of taking photographs)

OIL SPILL BIOREMEDIATION

Safety



Wear safety goggles, gloves, and a lab apron at all times while conducting this investigation.

Read all the instructions for this laboratory activity before beginning. Follow the instructions closely, and observe established laboratory safety practices, including the use of appropriate personal protective equipment (PPE).

Tetrazolium may cause skin and eye irritation. Rid-X® Septic System Treatment contains bacterial spores and enzymes and may cause lung irritation. Avoid contact with skin and eyes. Avoid breathing dust particles.

Household bleach can damage eyes and skin, and it should not be ingested. It should be used only in a well-ventilated area, such as a room with an open window or a bathroom with a ventilation fan. Always wear PPE (goggles, an apron, and gloves) when handling bleach. If bleach gets into your eyes, rinse them thoroughly with water for several minutes. If you are wearing contact lenses, remove them if practical to do so and then continue rinsing. If bleach contacts your skin, wash thoroughly with soap and water. If bleach contacts your clothing, remove and wash it before wearing it again. If ingested, do not induce vomiting. Rinse your mouth thoroughly with water, and seek medical attention immediately.

Do not eat, drink, or chew gum while performing this investigation. Wash your hands with soap and water before and after the investigation, and sanitize the work space with a 10% bleach solution after finishing. Keep pets and children away from lab materials and equipment.

Preparation

This will require 3 days of data collection once the experiment is set up. Please plan accordingly.

Many factors can affect the speed with which microbes break down substrate (i.e., temperature, salinity, availability of limiting nutrients, exposure to sunlight, and access to the substrate). In this activity, you will design an experiment to test the effects of one of these factors on the breakdown of oil using materials from your environment. Two tubes are provided in your kit to perform your test.

1. Read through the activities.
2. Obtain all materials.
3. Begin preparing the microbial suspension as follows:
   a. Using the graduated cylinder, measure out 140 mL of warm tap water in a plastic cup.
   b. Add the powdered contents of the Rid-X® Septic System Treatment container (~20 g) to the cup, and mix thoroughly by gently swirling the cup.
   c.  Let the mixture sit undisturbed for about 15 minutes to allow undissolved matter to settle.

After the undissolved matter has settled in the cup, continue preparing the microbial suspension, as follows:

   d. Fold the cheesecloth in half to double it. Place the folded cheesecloth in a funnel in the top of a second cup.
   e. Slowly pour the Rid-X® mixture into the cheesecloth in the funnel to filter the pulp from the microbial suspension.
   f. Dispose of the cheesecloth and pulp.

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4. To prepare the tetrazolium solution, pour the 100 mL of ***distilled*** water from the enclosed bottle into the enclosed brown bottle containing the tetrazolium powder. **Be sure to do this slowly.** Shake the brown bottle well to ensure the powder dissolves into solution.

ACTIVITY 1

Bioremediation of Oil

1. Use a pencil to label the culture tubes 1 through 6.
2. Tubes 1 and 2 will utilize the tetrazolium indicator to determine if metabolism is taking place. Hypothesize whether you think the indicator will change color in both tubes, neither one, or just tube 1 or tube 2, and describe your reasoning. Use Figure 3 to help form your hypothesis. Record this information in the "Hypotheses" section in your **Lab Worksheet**.
3. Tubes 3 and 4 will be used to examine the change in appearance and physical properties of the oil in the presence of microorganisms. Hypothesize if you will be able to see the oil broken down in either of these tubes without the indicator present, and describe your reasoning. Use Figure 3 to help form your hypothesis. Record this information in the "Hypotheses" section in your **Lab Worksheet**.
4. Tubes 5 and 6 will be used to test the effects of your chosen environmental factor on the breakdown of oil by microorganisms. Choose ***one*** of the following as your environmental factor to alter (please check with your instructor if you would like to use a different option): amount of microbes present, amount of oil present, type of oil present, light conditions, or temperature variation. Hypothesize which of the two tubes will experience the greater breakdown by microbes, and describe your reasoning. Use Figure 3 to help form your hypothesis. Record this information in the "Hypotheses" section in your **Lab Worksheet**.

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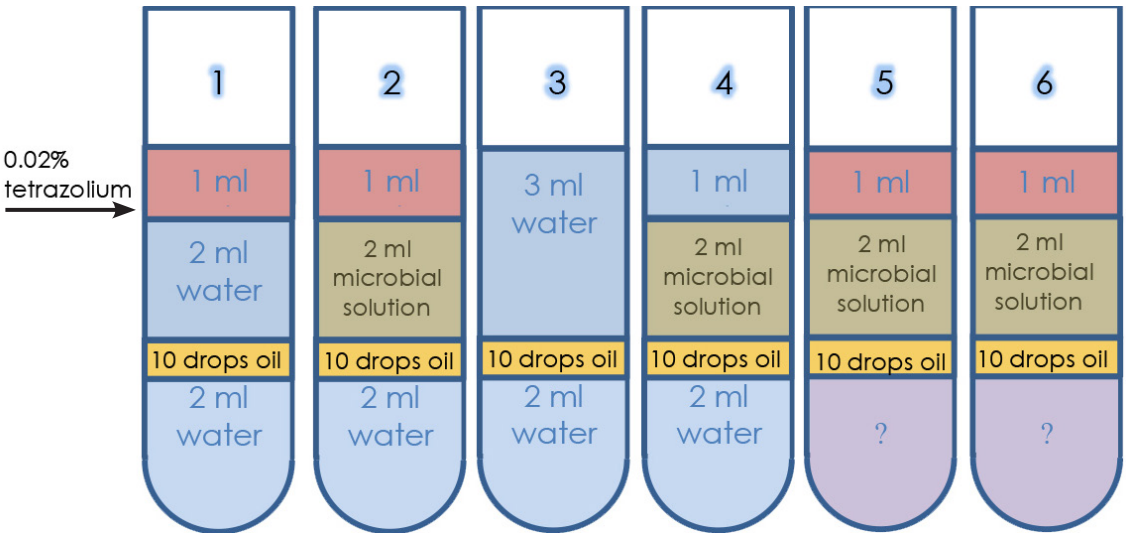
ACTIVITY 1 continued

5. Using a plastic pipet, add 1 mL of 0.02% tetrazolium indicator (made in the "Preparation" section) to tubes 1, 2, 5, and 6 only. Use the graduations marked on the plastic pipet to measure 1 mL. Discard the pipet when finished. (See Figure 3 for guidance in adding quantities.)
6. Using a clean plastic pipet, add 2 mL of distilled water to tubes 1, 2, 3, and 4 only. Use the graduations marked on the plastic pipet to measure 2 mL. Discard the pipet when finished.
7. Using a clean plastic pipet, add 10 drops of oil to all six tubes. Discard the pipet when finished.
8. Using a clean plastic pipet, add an additional 2 mL of distilled water to tube 1, an additional 3 mL to tube 3, and an additional 1 mL to tube 4. Use the graduations marked on the plastic pipet to measure each amount. Discard the pipet when finished.
9. Using a clean plastic pipet, add 2 mL of microbial suspension to tubes 2, 4, 5, and 6 only. Use the graduations marked on the plastic pipet to measure 2 mL. Discard the pipet when finished.
10. Add the required component(s) or set up the required changes for your experimental design to tubes 5 and 6, and record what you did in Data Table 3 of the "Observations/Data Tables" section of the **Lab Worksheet**.
11. Cap all culture tubes. Mix the liquid in all six tubes by finger vortexing them, one tube at a time. To finger vortex, hold the top of the tube securely in one hand; draw the index finger of the other hand toward you several times, gently tapping the side of the tube near the bottom. This creates a whirlpool inside the tube, which mixes the liquids. Repeat this procedure with the remaining tubes. Place all six tubes upright in a test tube rack.

Finger Vortexing
https://players.brightcove.net/17907428001/HJ2y9UNi_default/index.html?videoId=4573398312001

Figure 3.



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12. Record observations about the color, viscosity, and general appearance of the oil, today and for the following three days, in the corresponding data tables in the **Lab Worksheet**. Also, take photographs all four days. The photographs should show all 6 tubes (with their labels clearly visible) as well as a strip of paper with your name and the date written on it. You will be uploading these photographs to your lab report. Use the following tips to help clearly distinguish the differences between the tubes:

    a. To aid in your observations, finger vortex the tubes daily, as described in Step 11.

    b. Manipulate the tubes in any way that allows you to better view the characteristics of the oil in each.

    c. In particular, to aid in your observations of tubes 3 and 4, hold them up to the light in a horizontal position and observe how the oil moves over the liquid.

    d. Invert tubes 3 and 4 several times and watch the oil gather back at the top of the liquid. Observe any differences in the composition of the oil.

13. ***Gently*** loosen each cap on each culture tube by turning it 45 degrees counterclockwise. Let the tubes sit overnight.

Just before observing the tubes each day, retighten the caps on the tubes. After observing the tubes, loosen the caps again as explained in Step 13.

Submission

Using the **Lab Report Template** provided, submit your completed report to Waypoint for grading. It is not necessary to turn in the Lab Worksheet.

Disposal and Cleanup

1. Dispose of solutions down the drain with the water running. Allow the faucet to run a few minutes to dilute the solutions.
2. Rinse and dry the lab equipment from the equipment kit, and return the materials to your equipment kit.
3. Dispose of any materials from the materials kit in the household trash. The plastic funnel may be recyclable.
4. Sanitize the work space with a 10% bleach solution, and wash your hands thoroughly.

    Disinfecting a Surface
    https://players.brightcove.net/17907428001/HJ2y9UNi_default/index.html?videoId=4573412195001

ACTIVITY

Lab Worksheet

Hypotheses

Activity 1.

Tubes 1 and 2:

Tubes 3 and 4:

Tubes 5 and 6:

continued on next page

Observations/Data Tables

Data Table 1.

Tubes 1 and 2 Observations		
Day	**Tube 1**	**Tube 2**
0 (Initial setup)		
1		
2		
3		

Data Table 2.

Tubes 3 and 4 Observations		
Day	**Tube 3**	**Tube 4**
0 (Initial setup)		
1		
2		
3		

ACTIVITY

Data Table 3.

Tubes 5 and 6 Observations		
Chosen environmental factor to change:		
Day	**Tube 5**	**Tube 6**
0 (Initial setup)		
1		
2		
3		

NOTES

ENVIRONMENTAL SCIENCE
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