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Cleaning Up Oil Spills With Oil-Hungry Bacteria

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Objectives

- ***Learn*** **how naturally occurring microorganisms break down oil.**
- ***Model*** **various oil spill scenarios.**
- ***Test*** **the ability of naturally occurring microorganisms to break down oil.**
- ***Observe*** **the physical changes of oil during biodegradation.**

Background

In an increasingly technological society, petroleum products have become essential to maintain our high standard of living. Products derived from petroleum heat our homes, fuel our automobiles, and provide energy for running industrial plants. Because of the high demand for petroleum products, they must be stored and transported in large volumes. As a result of exploration activities or during storage or transport, petroleum products are sometimes spilled on land or into waterways. When this occurs, human health, plant life and living organisms in the surrounding area may be at risk.

Oil spilled at sea poses a serious threat to the marine environment. It has the potential to effect every level of the marine food chain. The marine environment is made up of complex interrelations between plant and animal species and their physical environment. Harm to the physical environment will harm one or more species in a food chain, which may lead to damage to other species further up the chain. Floating oil contaminates plankton, which includes fish eggs and larvae of various marine invertebrates. In turn, as the small fish feed on the plankton, they too become contaminated. Larger fish, land animals and even humans may eat these contaminated fish.

A single gallon of spilled oil can spread over 4 acres of water. The oil spreads horizontally into a smooth and slippery surface, called a "slick", which floats on top of the water. As soon as an oil spill occurs, natural actions take over which reduce the severity of the oil spill. These actions include weathering, evaporation, oxidation, biodegradation, and emulsification. However, natural actions alone are not enough to contain and treat most oil spills. Swift human intervention is needed to prevent oil spills from causing widespread damage to the environment.

Several methods of containing and cleaning up oil spills in an aquatic environment have been developed. Mechanical equipment, such as booms and skimmers are used to contain and recover the spilled oil. Chemical agents are used in place of, or in addition to, mechanical means. These agents disperse the oil slick, allowing natural processes - such as wind, waves, and currents - to break it down further. Biological agents have also been used in recent oil spills helping to increase the rate at which natural biodegradation occurs. Biodegradation is a process by which microorganisms such as bacteria, fungi, and yeasts break down complex compounds into simpler products to obtain energy and nutrients. These "oil-hungry" microorganisms convert oil into food for themselves. In the process, they convert the oil into non-toxic components, which then get safely assimilated into the aquatic food chain.

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In recent years, bioremediation techniques have been developed which can help biodegradation processes work faster. These techniques involve the addition of nutrients or microorganisms that increase the rate at which natural biodegradation occurs. The addition of nutrients such as phosphorus and nitrogen stimulate the growth of indigenous microorganisms capable of biodegradation. The addition of specific microorganisms to the existing native population can also be used to increase the population of microorganisms that can biodegrade oil spills naturally.

Bioremediation techniques have been used with some success in recent oil spills, including the infamous Exxon Valdez spill in 1989. Studies from this and other treatments have shown that the use of bioremediation more than doubles the rate of oil degradation and produces no harmful effects to the shoreline or sensitive habitats.

Safety

Although the oil-hungry bacteria that you'll be using in this investigation are naturally occurring and non-pathogenic, you should follow proper general lab safety and aseptic technique when working with any bacterial cultures or chemicals in the lab. Be sure to wear protective gloves, goggles and a lab apron.

Do not touch your face or mouth with your hands. Wash your lab table and your hands immediately after handling bacteria, oil or the stain provided in this kit. Keep your work area clean. Disinfect your area and dispose of all materials as instructed by your teacher.

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Activity 1

Biodegradation of an Oil Slick

What you need

(Per student)

Apron
Gloves, protective
Goggles

(Per group)

5 mL Oil-hungry microbial suspension

1 Magnifying glass

0.5 mL Motor oil, refined

1 Pipet, plastic

1 Stirrer, plastic

2 Vials, with caps

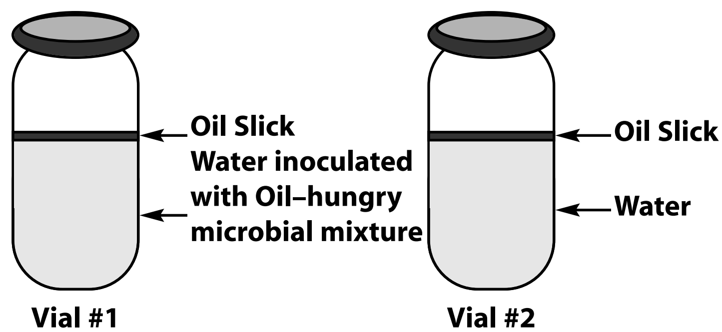
What to do...

Safety

Wear safety goggles, apron, and protective gloves.

STEP 1

Using a wax pencil, label two vials "#1" and "#2". Fill each vial half way with distilled or tap water.



STEP 2

Add 2-3 drops of refined motor oil to each vial of water - just enough oil to form a thin layer.

STEP 3

Caution: *Although these organisms are non-pathogenic, you should follow proper aseptic technique when using any bacterial cultures in the lab.*

Stir the oil-hungry microbial suspension provided by your teacher so that all of the solid particles are evenly suspended in solution. Using a pipet, add 5 mL of the oil-degrading microbial solution, drop by drop, to the top of the oil film in Vial #1 only. Be sure to add the solid particles suspended in the microbial solution on the oil slick as well. What is the purpose of vial #2, since it was not inoculated with the oil-hungry bacteria?

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STEP 4

Place the cap on each vial and carefully turn the vial upside down a couple of times to mix the contents. Why is this necessary?

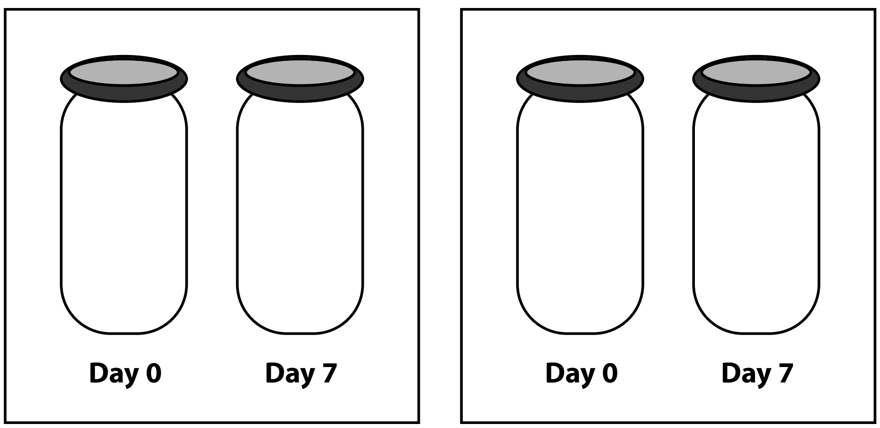
Illustrate and describe below the initial physical characteristics - color, texture, viscosity, oil dispersion, and overall appearance - of the oil slick and turbidity of the water in both vials. Record your observations in Table 1.

STEP 5

Loosen the cap on each vial, and place both of them in the location your teacher directs.

STEP 6

Disinfect your area, as your teacher directs, and wash your hands thoroughly before leaving the laboratory. At the end of the lab activity, be sure to dispose all of the materials as your teacher directs.



Vial #1 Vial #2

Recording Observations

1. Use a magnifying glass to observe any changes that may take place in each vial over 5-7 days, as your teacher directs. Carefully note observed changes in Table 1 and illustrate your observations on the last day of the experiment. Each day, after you make your observations, temporarily place the cap on Vial #1 and invert it once or twice to increase the dissolved oxygen content and to mix the microbes with the oil.

2. What happens to the oil slick over time? Can you observe signs of the oil film breaking down (degrading) in either vial? Do you think there is a correlation between the turbidity of the water, growth rate of bacteria and the amount of oil degraded?

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Data Table #1

Day	Vial #1 Description of Oil Slick (texture, color, viscosity, consistency)	Vial #1 Description of Water (Turbidity)	Vial #2 Description of Oil Slick (texture, color, viscosity, consistency)	Vial #2 Description of Water (Turbidity)
0 (Start)				
1				
2				
3				
4				
5				

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Questions

1. Which of the two vials is the control? Why is it necessary to have a control?

2. Based upon your observations write a definition for biodegradation.

3. Based upon your observations describe a predictable pattern of oil biodegradation.

Day	Appearance
1	
2	
3	
4	
5	

4. Based on your direct observations, what do you suppose is happening to the degrading oil molecules?

5. What happens to the bacteria after it consumes the oil and to the remaining oil that does not get consumed by the bacteria in nature?

6. What variables could effect the degradation of an actual oil slick?

7. What does an increase in turbidity in the water in your test vial indicate?

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Activity 2

Cleaning Up Mini Oil Spills in Various ShoreEnvironments

What you need

(Per student)

- Apron
- Gloves, protective
- Goggles

(Per group)

	Cobbles
1	Magnifying glass
1 mL	Motor oil, refined
10 mL	Oil-hungry microbial suspension
1	Petri dish, three-chamber
1	Pipet, plastic
	Sand, coarse
	Sand, fine
1	Stirrer, plastic

What to do...

Safety

Wear safety goggles, apron, and protective gloves. Although these organisms are naturally occurring and non-pathogenic, you should follow proper aseptic technique when working with any bacterial cultures in the lab.

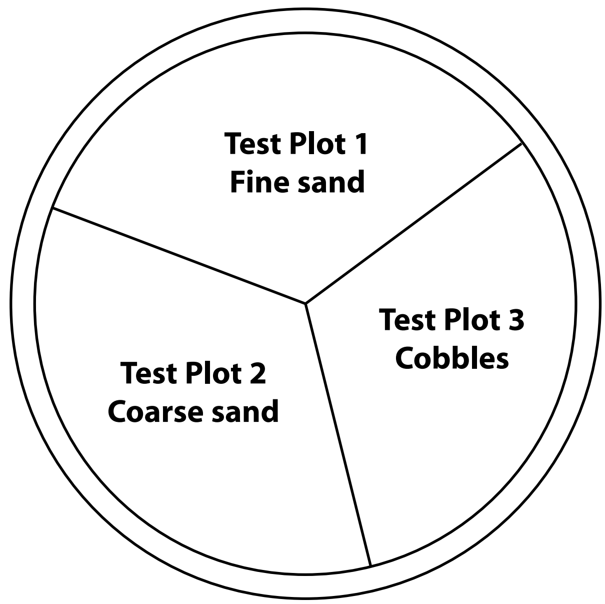
STEP 1

Use the marking pencil to label each chamber of your petri dish as follows:

Test Plot #1: "Fine sand"

Test Plot #2: "Coarse sand"

Test Plot #3: "Cobbles"



STEP 2

Create three artificial beaches by filling each individual chamber of your petri dish with a corresponding type of beach material: fine sand, coarse sand or small cobbles (rounded, flat stones). Obtain each of these beach materials from your teacher.

STEP 3

Using a plastic pipet, carefully add 2-3 drops of oil over the "fine sand beach" portion of your dish. This should be added near the outside perimeter so that the oil is visible from the side on the dish as it permeates the beach material.

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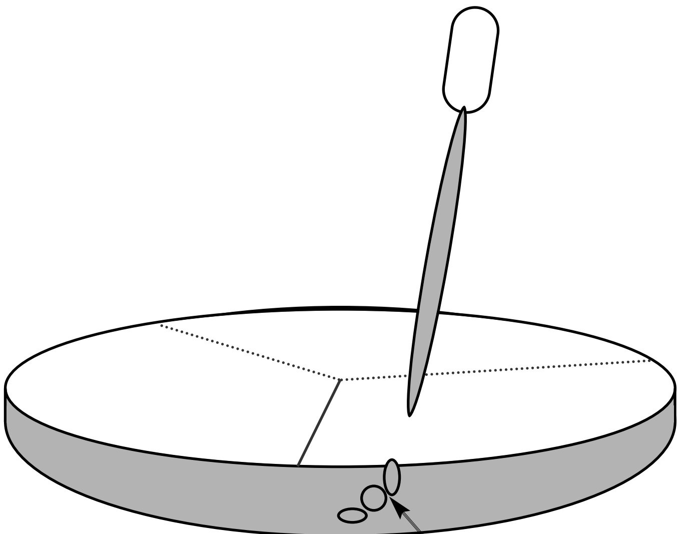

Observe what happens to the oil. Is it readily absorbed by the fine sand? Time how long it takes for the oil to reach the bottom of the dish.

STEP 4

Repeat Step #2, using the remaining two beach types on your dish, coarse sand and cobbles.

Which of the three beach materials allows the oil to permeate at a faster rate? What do you think would happen if there was an oil spill in beach environments similar to your beach material? Which beach material would contain oil spills better?

STEP 5

Stir the oil-hungry bacteria suspension so all the solid particles are evenly suspended in solution.Using a plastic pipet, apply approximately 5 mL of the oil-degrading microbial mixture, drop by drop on each test plot, just enough to form a thin layer over the beach material. Be sure to add the solid particles suspended in the microbial solution on the oil slick.

STEP 6

Using a plastic pipet, apply 5-6 drops of oil on each test plot to create an oil slick.

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STEP 7

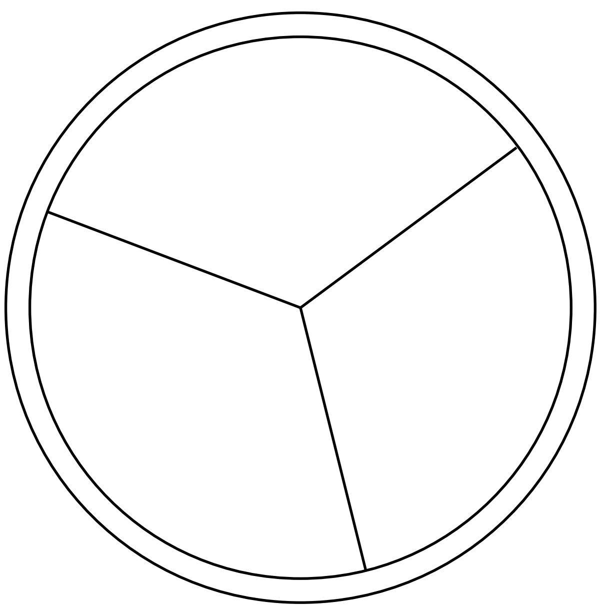
Illustrate and describe below the initial physical characteristics - color, texture, and overall appearance - of the oil slick on each test plot. Record your observations in Table 2.

STEP 8

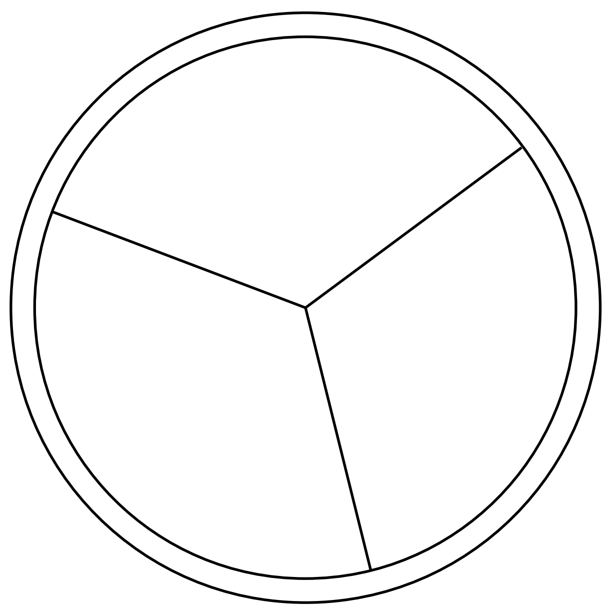
Cover your dish and store it in a warm spot in the laboratory as your teacher directs.

STEP 9

Disinfect your area as your teacher directs and wash your hands thoroughly before leaving the laboratory. At the end of the lab activity, be sure to dispose all of the materials as your teacher directs.



Day O



Day 7

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Recording Observations

1. Use a magnifying glass to observe any changes that may take place in each test plot over 5-7 days, as your teacher directs. Be sure to leave the cover on the petri dish while making your observations. Carefully note observed changes in Table 2, and illustrate your observations on the last day of the experiment.

2. What happens to the oil slick over time? Can you observe signs of the oil slick breaking down (degrading) in each test plot? Do you think there is a correlation between growth rate of the bacteria and the amount of oil degraded?

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Data Table #2

Day	**Test Plot 1 (Fine Sand) Description of Oil Slick** (texture, color, consistency, overall appearance)	**Test Plot 2 (Coarse Sand) Description of Oil Slick** (texture, color, consistency, overall appearance)	**Test Plot 3 (Cobbles) Description of Oil Slick** (texture, color, consistency, overall appearance)
0 (Start)			
1			
2			
3			
4			
5			

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Questions

1. How effective was the application of the oil-hungry bacteria on each of the test plots? Was there more or less oil degradation and bacterial growth in each of the test plots? Compare data from other groups. How effective was the application of product in attacking subsurface oil?

2. Environmentalists may argue that the addition of fertilizers to enhance indigenous bacterial growth or the seeding of actual bacteria of an oil spill poses a greater risk than the oil itself. What are your views on this statement?

3. What are some benefits to using bioremediation to clean up oil spills, as opposed to using chemical or mechanical techniques?

4. Why was there still oil remaining in the petri dishes, even after 5 days of biodegradation?

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ACTIVITY 3

Microscopic Observations (optional)

What you need

(Per group)

	Methylene blue stain
1	Microscope slide
1	Microscope slide coverslip
1	Pipet, plastic
	Oil, biodegraded, from a test plot
	Oil, untreated

What to do...

Safety

Wear safety goggles, apron, and protective gloves. Although these organisms are naturally occurring and non-pathogenic, you should follow proper aseptic techniques when working with any bacterial cultures in the lab.

STEP 1

Place a small drop of the biodegraded oil from one of the Test Plots on a microscope slide and spread it into a very thin layer. Add a drop of methelyne blue stain over your sample and cover it with a coverslip.

STEP 2

View the microscope slide under a compound microscope, first at low magnification and then at high magnification. Note the texture of the remaining oil and any bacterial cells surrounding it.

STEP 3

Repeat Steps 1 and 2 using a drop of untreated oil. Compare the physical characteristics of the untreated oil to the biodegraded oil. Record your observations in Table 3.

STEP 4

Disinfect your area as your teacher directs and wash your hands thoroughly before leaving the laboratory. At the end of the lab activity, be sure to dispose all of the materials as your teacher directs.

Recording Observations

Data Table #3

OBSERVATIONS	
Biodegraded Oil	**Untreated Oil**

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Going Further

1. Design your own experiments to test the following hypotheses:

  - Lower temperatures slow down bacterial growth so that oil degradation would take longer.
  - Higher temperatures would inhibit bacterial growth and thus slow down spill degradation.
  - Increased wave action (agitation) would increase the amount of oxygen that is mixed in with the oil slick, aid in microbial growth and increase the rate of oil degradation.
  - Sunlight alone would break down part of the oil slick, through a process called photolysis - decomposition of a compound into simpler units through exposure to light.

2. Have students compare this bioremediation cleanup measure (biodegradation) with the application of chemical agents on similar simulated beach oil spill scenarios. Determine which method is more effective. Chemical agents include citrus oil cleaners (green detergents), household washing detergents, and detergents having special enzyme cleaners.

3. Research ways to clean up oil spills on land and waterways. Do library research or search the Internet to find out how effective bioremediation has been in cleaning up oil spills.

Learn & Read More About It

Alexander, Martin (Editor). *Biodegradation and Bioremediation*. Academic Press, 1994.

Lord, Nancy. *Darkened Waters: A Review of the History, Science, and Technology Associated With the Exxon Valdez Oil Spill and Cleanup*. Homer Society of Natural History, 1992.

Rozens, Aleksanders. *Environmental Destruction (When Disaster Strikes)*. Twenty FirstCentury Books, 1995.

Skipper, H.D. (editor). *Bioremediation: Science and Applications*. American Society of Agronomy, 1995.

Neat Websites

Provides thorough information about oil spills, ways to remediate them and prevention methods.
www.epa.gov/oilspills

Complete database of oil spills
http://www.cutter.com/oilspill/reports/vessels.htm

Provides information about the Valdez Oil Spill
http://www.planetdiary.com/background/oils.html

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