Neo/SCI™
Teacher's
Guide

Neo SCI®

New ideas for teaching science

WARNING — This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision.

Cleaning Up Oil Spills With Oil-Hungry Bacteria

INVESTIGATION

#20–1253

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**

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New ideas for teaching science

Science Concepts

- Bacteria
- Biodegradation
- Bioremediation
- Ecosystem
- Food chain

Kit Materials List

	Methylene blue stain, 1%
	Cobbles
100	Gloves, disposable
5	Magnifying glasses
20	Measuring cups, plastic
	Measuring spoon
30 mL	Motor oil, refined
5 g	Oil-hungry bacteria blend (non-pathogenic)
20	Petri dishes, 3-chamber
20	Pipets, plastic
	Sand, coarse
	Sand, fine
20	Stirrers, plastic
20	Vials, with caps

Time Requirements

Pre-Lab Preparation
10 minutes (2 hours before the investigation)

Lab Activity 1
30 minutes for first day and 10 minutes each day thereafter

Lab Activity 2
30 minutes for first day and 10 minutes each day thereafter

Lab Activity 3
15 minutes

Safety & Disposal

Although the oil-hungry bacteria that students will use in this investigation are naturally occurring and non-pathogenic, have students follow proper lab safety and aseptic technique protocols. Students should wear protective gloves, goggles and a lab apron when working with bacteria or any chemicals. Be sure that students do not touch their face or mouth with their hands. Instruct your students to disinfect their work area and wash their hands immediately after handling bacteria.

At the end of the investigation, collect all petri plates and all contaminated items such as pipets, slides, etc. Prior to disposal, sterilize all contaminated materials by immersing them in 5% bleach solution for about 1 hour. The work area should be decontaminated by wiping it with either a bleach solution or 95% ethanol.

Pre-lab Setup

Included in this kit is a packet of specially blended oil-hungry bacteria mixed with nutrients that have been successfully used on actual oil spills. These microbes are non-pathogenic and have been selected for their ability to degrade refinery and petroleum wastes in marine environments.

The culture is a tan, free flowing powder, with a yeast-like odor. It contains over 5 billion bacteria cells per gram! These microbes work in a pH range of 6.0-8.5 and at a temperature range of 50-110ºF. Point out to students that in an actual spill situation, the most likely contaminant is crude oil. The oil that they will use in this investigation is a refined product derived from crude oil. Crude oil may be substituted in this activity if it is available locally.

Caution: *Have your students follow proper aseptic technique when working any bacterial cultures. They should avoid creating dusts.*

At least 2 hours prior to the investigation, soak the oil-hungry bacterial mixture at a ratio of 2 g (approximately 1 teaspoon) to 150 mL of lukewarm water for about 2 hours, stirring occasionally. Use the measuring spoon provided to weigh out the bacteria mixture.

Dispense 5 mL of the bacterial suspension per group for Activity 1 and 15 mL of suspension per group for Activity 2.

Enough materials are provided for up to 40 students working in groups of 4 in Activity #1 and groups of 2 in Activity #2.

Using the materials provided in this kit, students may conduct other inquiry-based activities. They should begin with a question, design their own experiment, gather and analyze data, formulate a conclusion to support the original solution, and present their results in a scientific manner.

Lab Activity Procedures & Notes

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

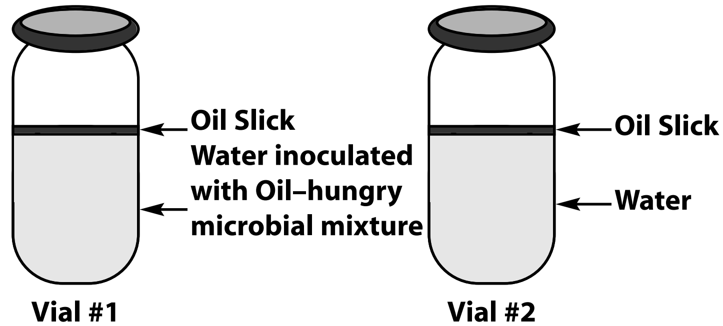
What to do . . .

Safety

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

STEP 1

Using a wax pencil, your students should label two vials "#1" and "#2". They should fill each vial half way with distilled or tap water.



STEP 2

Next, they should 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, students should follow proper aseptic technique when using any bacterial cultures in the lab.*

They should stir the oil-hungry microbial suspension, so that all of the solid particles are evenly suspended in solution. Using a pipet, they should 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 they add the solid particles suspended in the microbial solution on the oil slick. What is the purpose of vial #2, since it was not inoculated with the oil-hungry bacteria?

Vial #2 will be used as the control.

STEP 4

The students should 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?

This step helps to intermix the bacteria with the oil and provides oxygen for the bacteria to grow. In nature, this would occur by wave action, wind and other physical forces.

Students should 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. They should record their observations in Table 1.

Day 0 Day 7

STEP 5

Instruct students to loosen the cap on each vial and store them in a warm spot in the laboratory.

STEP 6

They should disinfect their area at your direction and wash their hands thoroughly before leaving the laboratory. At the end of the lab activity, be sure they dispose all of the materials as you direct.

Observations

1. Have your students use a magnifying glass to observe any changes that may take place in each vial over 5-7 days. They should carefully note observed changes in Table 1 and illustrate their observations on the last day of the experiment. Each day, after they make their observations, they should 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?

   The oil should start showing signs of degradation after 2-3 days of microbial action. The color of the oil should change from a light brown to tan/brown color. The continuous oil layer that covers the water surface at the start should start to disperse into smaller globs and fine droplets.

   A turbid water solution indicates an increased bacterial growth as well as oil degradation, as the oil is dispersed and small droplets suspend in the water column.

   The control jar should show very little change in terms of how the oil layer looks due to the mixing of water with it. However, there should be a drastic difference in oil and water layers in Vial #1 which was inoculated with the oil-hungry bacteria compared to Vial #2.

Recording Observations

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)	Free-flowing, light brown	Clear	Free-flowing, light brown	Clear
1	Free-flowing, light brown	Clear	Free-flowing, light brown	Clear
2	Oil is turning globular, light brown mixed with light tan spots	Slightly turbid	Free-flowing, light brown	Clear
3	Oil continues to turn into more viscous globs, light brown mixed with light tan spots	Slightly turbid	Free-flowing, light brown	Clear
4	Oil is starting to disperse into small droplets, tan color	Increasing turbidity	Free-flowing, light brown, with some globs	Clear
5	Most of the oil is dis-persed, tan color, frothy appearance	The turbidity continues to increase	Free-flowing, light brown, with some globs. Some breakdown of the oil will occur as it mixes with the water.	Clear

Note: *The sample results provided in Table 1 are an illustration of the pattern students should observe with respect to the oil degradation.*

New ideas for teaching science

Questions

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

   Vial #2 is used as the control in this investigation. A control is used as a comparison to the variable being tested in an experiment.

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

   Answers will vary. 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.

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

Day	Appearance
1	**Color change from light brown to tan**
2	**Globular in appearance**
3	**Oil is dispersed in fine droplets**
4	**Increase in turbidity of the water surrounding it**
5	**Frothy in appearance**

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

   Bacteria converts the complex oil molecules into simpler products to obtain energy and nutrients. These "oil-hungry" microorganisms convert oil into food for themselves, and in the process they convert the oil into nontoxic components.

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?

   The population of the oil-hungry bacteria in nature would start to die out and decrease after it runs out of oil which is used as their primary carbon source. The remaining oil components get assimilated into the aquatic food chain.

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

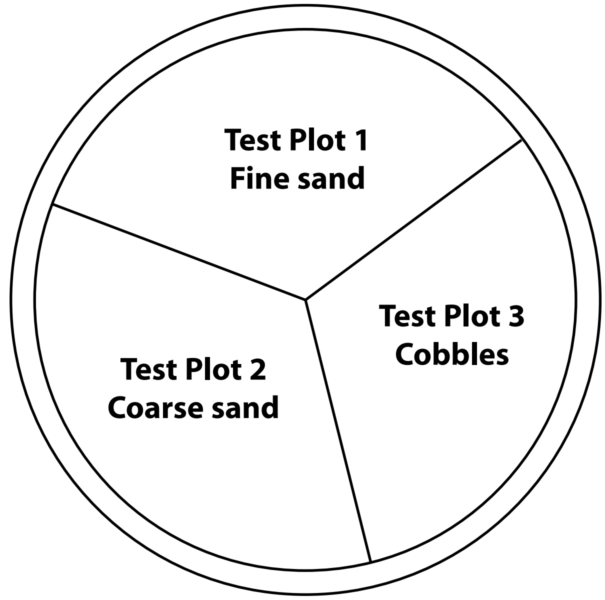
   Variables that effect the rate of oil degradation include wave action (physical mixing), sunlight, temperature and indigenous microbial population adapted to use petroleum products as a source of carbon.

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

Lab Activity Procedures & Notes

Activity 2

Cleaning Up Mini Oil Spills in Various Shore Environments



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

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

STEP 1

They should use the marking pencil to label each chamber of their petri dishes as follows:

Test Plot #1: "Fine sand"

Test Plot #2: "Coarse sand"

Test Plot #3: "Cobbles"

STEP 2

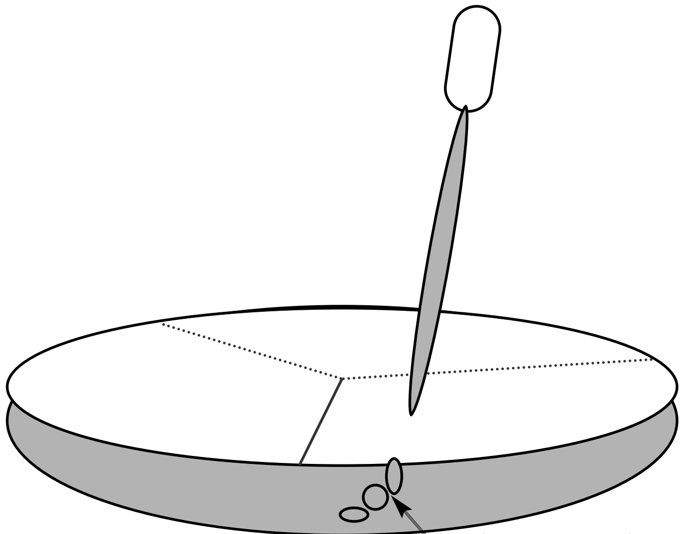
They should create three artificial beaches by filling each individual chamber of their petri dishes with a corresponding type of beach material: fine sand, coarse sand or small cobbles (rounded, flat stones) which they obtain from you.

STEP 3

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

Students should 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.

Because of the low porosity of the fine sand, the oil permeates the sand slowly and is absorbed completely in about 3-4 seconds.



STEP 4

They should repeat Step #2, using the remaining two beach types on their dishes, 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?

The cobbles have the greatest porosity capacity compared to the fine and coarse sand, allowing the oil to permeate at a much faster rate. An actual oil spill in a cobble beach would make it difficult to remediate the site using traditional mechanical means, since most of the oil would flow through the porous layer making it difficult to reach. Oil spills are contained more easily on non-porous earth layers.

STEP 5

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

STEP 6

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

STEP 7

Students should illustrate and describe the initial physical characteristics - color, texture, and overall appearance - of the oil slick on each test plot. They should record their observations in Table 2.

STEP 8

Instruct your students to cover their dishes and store it in a warm spot in the laboratory.

STEP 9

Instruct your students to disinfect their area and wash their hands thoroughly before leaving the laboratory. At the end of the lab activity, be sure they dispose of all of the materials at your direction.

Observations

1. Students should use a magnifying glass to observe any changes that may take place in each test plot over 5-7 days. As a safety precaution, instruct students to leave the cover on the petri dish while making their observations. Under your supervision, you may want to remove the cover temporarily so students can get a better view of the oil degradation process. They should carefully note observed changes in Table 2, and illustrate their 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?

   Over time the oil slick gets surrounded by the oil-hungry bacteria as they feed on it. Signs of oil degradation include color, texture and overall change of appearance. The bacterial growth rate would increase as more oil is used by the bacteria as their primary source of carbon.

Day 7

Recording Observations

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)	Free-flowing, light brown	Free-flowing, light brown	Free-flowing, light brown
1	Free-flowing, light brown	Free-flowing, light brown	Free-flowing, light brown
2	Oil is turning globlular, light brown mixed with light tan spots	Oil is turning globlular, light brown mixed with light tan spots	Light tan globs of degraded oil surround the pebbles.
3	Oil-hungry bacteria has surrounded the oil slick, which now appears completely tan in color	Oil-hungry bacteria has surrounded the oil slick, which now appears completely tan in color	Oil continues to change in appearance, viscosity and color.
4	The growth of bacteria continues to increase and break up the oil into a frothy-looking material.	The oil appears light tan and frothy.	The oil appears light tan and frothy.
5	The growth of bacteria continues to increase and break up the oil into a frothy-looking material.	The oil appears light tan and frothy.	The oil appears light tan and frothy.

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?

   As evidenced by the bacterial growth and the physical changes to the oil, there should have been considerable oil degradation. The cobble stone test plot should have more bacterial growth, due to the cobbles providing a greater surface area, as well as more oxygen for the bacteria to grow as compared to the fine and coarse sand test plots.

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?

   Answers will vary based on students' knowledge of the process and beliefs.

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

   Answers will vary. Bioremediation techniques has been used with some success in recent oil spills, including the 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 produced no harmful effects to the shoreline or sensitive habitats.

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

   The process of bioremediation is a very slow process. The bacteria applied on your simulated oil spill cannot degrade the oil completely by itself. In nature it uses certain portions of the oil as a source of carbon and converts the rest of it into less harmful components which are then assimilated into the food chain by other organisms. In addition, actual oil spills get a head start from natural physical forces such as waves, wind, and the sun.

New ideas for teaching science

Lab Activity Procedures & Notes

Activity 3

Microscopic Observations (optional)

What you need

(Per group)

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

What to do . . .

Safety

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

STEP 1

Students should place a small drop of the biodegraded oil from one of their test plots on a microscope slide and spread it into a very thin layer. They add a drop of methelyne blue stain over your sample and cover it with a coverslip.

STEP 2

Next, they view the microscope slide under a compound microscope, first at low magnification and then at high magnification. They should note the texture of the remaining oil and any bacterial cells surrounding it.

STEP 3

They now repeat Steps 1 and 2, using a drop of untreated oil. They should compare the physical characteristics of the untreated oil to the biodegraded oil and record their observations in Table 3.

STEP 4

At your direction, students should disinfect their area and wash their hands thoroughly before leaving the laboratory. At the end of the lab activity, be sure they dispose all of the materials as you direct.

Data Table #3

OBSERVATIONS	
Biodegraded Oil	**Untreated Oil**
Highly textured, and dispersed into fine droplets surrounded by bacteria.	**Smooth, free-flowing texture.**

Assessment

Assess students on their understanding of food chains and related ecological concepts.

Assess students on their knowledge of the scientific method by having them design a related experiment of their choice.

Assess students on their speaking and presentation skills during mock town meeting discussions related to oil spill issues.

Cross-Curricular Integration

Mathematics

Math concepts can be integrated with oil spill concepts. Have students apply their math skills to calculate the surface area for all three test plots in Activity 2, using the following formula:

$$A = \pi r^2$$

Based on this surface area and the amount of oil-hungry bacteria applied on this simulated oil spill, have them determine the quantity of oil-hungry bacteria that would be needed to remediate an actual oil spill covering 1 square kilometer.

Chemistry

The chemistry of various commercially available petroleum products can be discussed, including how they are refined and extracted from crude oil.

Environmental science

Related environmental issues, such as solid waste management and landfills, water pollution, air pollution, can be woven in with oil spill concepts.

Earth science

Discuss the various earth layers that make up the earth's crust, where crude oil is found and how it is originally formed.

Health

Discuss with students the various potential health risks associated with oil spills, even those which occur in other parts of the world.

Economics

Discuss with students the economic implications of oil spills, in terms of costs involved to remediate a site, as well as the adverse effects it has on the environment, living organisms and humans.

Social issues

Have students hold a mock town meeting to debate and present issues and concerns related to a nearby oil spill site. Students can represent concerned citizens in the area, waste management engineers representing the town, bioremediation specialists and company representatives whose employees caused the spill. Have students identify potential health concerns, ways to remediate the site, costs, ways to prevent any future spills and any long term economic implications due to a contaminated site