

## Oil-eating bacteria

An oil spill is an environmental hazard that is dangerous to many species of plants and animals. One of the methods of cleaning up oil spills that has been investigated is the use of oil-eating bacteria.

These strains of soil bacteria naturally use oils in the environment as their food. They also need some inorganic nutrients, oxygen and water in their environment in order to survive. The oil-digesting abilities of soil bacteria are thought to vary depending on the amount of oil found in the natural environment of different bacterial strains.

In this activity you will be working in groups to test this hypothesis.

If a whole class is doing the experiment, you should be able to test several different soil samples. Each sample is to be tested by at least two groups in the class. If you are doing this experiment by yourself, test at least two soil samples and repeat these tests.

One of the soil samples should be collected from an area already contaminated with oil. Some of the bacteria in this sample may be a very effective oil-degrading strain. Try collecting bacteria where engine oil often leaks on the ground (for example, service stations, dirt car parks). Another sample could be collected from an area with lots of leaf litter. Think of other different types of sites to test.

### You will need:

- four 500 ml beakers or glass jars
- aquarium pumps (one or two)
- four tubes to attach to the pumps
- splitter(s) to attach the tubes to the pump (one four-way, or two two-way)
- pipette or dropper
- brown paper about ten centimetres square (ten pieces)
- four petri dish halves (or aluminium foil) to act as a cover for the beakers. Drill a hole through the dishes to allow the tubing attached to the aquarium pump to pass through
- ammonium phosphate (0.5 g)
- magnesium sulfate (0.1 g)
- potassium phosphate (0.5 g)
- non-iodinated sodium chloride (2.5 g)
- lightweight machine oil (8 g)
- distilled water (600 ml)
- soil (10 g).

**What to do:**

1. Number the beakers from 1 to 4.
2. Put 150 ml distilled water and 2 g machine oil into each beaker. Put Beaker 1 aside.
3. Add half the inorganic nutrients to Beaker 2 (0.25 g ammonium phosphate; 0.05 g magnesium sulfate; 0.25 g potassium phosphate; 1.25 g non-iodinated sodium chloride).
4. Add half the soil sample (5 g) to Beaker 3.
5. Add the rest of the inorganic nutrients and the rest of the soil sample to Beaker 4.
6. Cover the tops of the beakers loosely with inverted petri dish halves, to reduce evaporation of the water and oil during the experiment.
7. Attach the tubes to the small aquarium pump. If you use a four-way splitter, only one pump will be needed for the four beakers.
8. Arrange the tubes so that they reach under the surface of the solution in each beaker and bubble air through the mixtures.
9. Carry out a grease spot test on each of the four beakers, every three days or weekly, for up to 30 days. The instructions for the grease spot test appear at the end of this sheet. When you do the first grease spot test, predict the trends that you might see in each test over the 30 days.
10. Convert your collected data into graphs and compare any changes in the results of the grease spot test over time in the four different beakers.
11. Analyse the experimental method and the data by considering the questions below.
12. Write a conclusion for the experiment.

**Analysis of the experimental method**

Consider the following questions.

- a) Which of the beakers was the experimental beaker and which were controls?
- b) Why was each of the controls needed?
- c) Why should each soil sample be tested by at least two groups in the class, or the tests repeated if you are working alone?

**Analysis of the data**

Consider each of the following questions.

- a) Was there a significant change in the results of the grease spot test in any particular tube?
- b) What could cause a change in the size of the oil spot over time?
- c) Did other groups get the same sorts of results?
- d) Do the results tell you anything about the oil-digesting abilities of the bacteria in the soil samples? If so, what?

**For further investigation**

Select the soil sample that has the most effective oil-digesting bacteria. Design an experiment to find out what conditions the bacteria work most effectively in. You could test temperature, light, nutrient concentration, oxygen levels, or any other physical condition you can think of.

**The grease spot test**

1. Divide one piece of brown paper into quarters. Number each of the four squares in the corner.
2. Using a pipette or dropper, draw a small volume of liquid from just under the top of the water level of a beaker.
3. Put three drops of this liquid into the centre of the correct square of paper (for example, if the sample was from Beaker 1, place the three drops on the square labelled 1). Take samples from the same place in each beaker each time.
4. Leave the paper to stand for a few hours. The water will evaporate, leaving a greasy spot in each square.
5. Draw a circle around the outside of each greasy spot with a pencil and measure and record the diameter of the spot.