SCIENCE IN THE REAL WORLD

Microbes in Action

Microbial Oil Degradation

Science in the Real World
Microbes In Action

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At a glance

Description
Students will investigate oil degradation by bacteria in drain cleaners.

Time Requirements
A Slick Oil Lab requires one class period to set up and results are visible within 1-5 days.

Curriculum Placement
This unit will fit into a microbiology unit, an environmental science unit or an enzyme unit. It could also be used to show scientific method and controlled experiments.

Materials and Equipment (for each lab group)
4 borosilicate culture tubes (18 x 150)
4 caps (18 mm)
1 test tube rack
marking pen
6 dropping pipettes (1 ml plastic)
0.02% tetrazolium indicator
drain cleaners (at least 3 kinds)
cooking oil (each group will test one kind)
label tape

Needed For Preparation:
2-3 weigh boats
balance
label tape or marking pen

A Slick Oil Lab

Introduction

Over the last 3 1/2 billion years microorganisms have evolved that can use almost every carbon compound as a food and energy source. Oils are long chain hydrocarbon molecules (that means that they contain only hydrogen and carbon) with a variety of side branches. Animal fats and vegetable oils are easily broken down by digestive enzymes in animals. Mineral oil and petroleum oil products (gasoline, diesel fuel, motor oil), cannot be digested or used as food by animals.

Many bacteria digest diverse types of oil, not only those of plant or animal origin, but also petroleum products. Scientists have taken advantage of the unique ability of microorganisms to work in tough environments. This has been especially useful for waste containing oil, which is difficult to dissolve and which causes other compounds to become stuck in the waste. While the material in a clogged drain looks disgusting to us, it actually contains many compounds that serve as carbon, nitrogen and energy sources for microorganisms. Mixtures of bacteria can digest the oil as well as many other nutrients in the waste, allowing the remainder of the waste to dissolve. This is the scientific basis for the “environmentally friendly” drain cleaners that you will study in lab today.

You will determine whether microorganisms in some brands of drain cleaners can metabolize vegetable oil. You will use an indirect method that gives visible results more quickly than observing either growth of the microorganisms or the disappearance of the oil, either of which could take several weeks.

The three components of the experiment are:

1. commercial drain cleaners that list bacteria as an ingredient

2. tetrazolium indicator solution (turns pink when oil degrading bacteria are active)

3. cooking oil

You will mix these ingredients and observe the tubes for about one week.

Based on the information you have:

1. Write a hypothesis concerning the drain cleaners and the oil. Record your hypothesis on your data sheet.

2. What results would support your hypothesis? Explain.

3. Design a control for this experiment. Describe and explain your control on your data sheet.

Materials

1 test tube rack
4 test tubes with caps
labels and/or marking pen
8 ml 0.02% tetrazolium indicator solution
3 ml drain cleaners (3 different brands)
2-3 ml cooking oil
6 dropping pipettes

Procedure

Prior to the day of the lab, bring a sample of cooking oil for your lab group (e.g. canola, corn, olive, peanut, safflower, or other vegetable oil). You will need only an ounce.

1. Write down the names of your drain cleaners and cooking oil on the data page. Mark 3 test tubes 1, 2, 3.

2. To each of the test tubes, add 2 ml tetrazolium indicator.

3. To each test tube add 5 drops cooking oil.

4. To test tube 1, add 2 ml of drain cleaner 1. To test tube 2, add 2 ml of drain cleaner 2. To test tube 3, add 2 ml of drain cleaner 3.

5. Cap the tubes and finger vortex each to mix.

6. Set up a control tube for this experiment.

7. Label your test tube rack and store as directed by your teacher.

8. Design a data table in which to record your observations daily for one week. Oil degradation will cause the tetrazolium indicator to turn pink.

9. Observe your set of tubes immediately after they are set up and record their appearance in your data table.

10. Observe your set of tubes daily for a week. Record any physical changes on your data table (on your data sheet).

Name ______________________________

Date _______________________________

Student Data Sheet

Name of drain cleaners:

1. _______________________

2. _______________________

3. _______________________

Cooking oil: _____________________________

1. Hypothesis: ____
   ____

2. What results would support your hypothesis? Explain. _________________________
   ____
   ____

3. Describe and explain your control. ____
   ____
   ____

4. Data table:

Drain cleaner name				
	Tube 1	***Tube 2***	***Tube 3***	***Control tube***
Day 0				
Day 1				
Day 2				

Analysis

5. Did all drain cleaners react the same? ______________________

   Which ones showed the most evidence of microbial metabolism? ________

   ____

6. Compare your results with classmates who used a different oil.

   Did all oils show evidence of microbial metabolism? ______________

   Explain your answer. ______________________________

   ____

7. Why do you think some bacteria might grow better with certain oils as a food source compared to other oils?

   ____

   ____

8. Find out what type of oil your family uses. Based on the results from this lab, which drain cleaner might work best if your kitchen drain became clogged? Explain your answer.

   ____

   ____

   ____

9. Do you think bacteria could degrade petroleum oil products? Would all bacteria be effective for treating oil spills?

   ____

Conclusion

Write a brief paragraph discussing your observations and conclusions reached in this activity.

____

____

____

Name ____________________________

Date ________________________

For Further Study

There are many household products that seem greasy or oily. These include things like butter, shortening, hand lotions, petroleum jelly etc. Will these drain cleaners work equally well on all oily materials? Design an experiment that will test this question. If time permits, try the experiment.

Teacher Guide

Instructional Objectives
At the end of this unit of activities the student should be able to:

1. demonstrate the methods of scientific inquiry by

    a. stating a problem
    b. writing a hypothesis
    c. performing an experiment according to given directions
    d. gathering data
    e. analyzing data
    f. developing further investigations

2. demonstrate the following laboratory skills:

    a. use a pipet to measure volume
    b. create a control
    c. finger vortexing
    d. design a data table

3. demonstrate the understanding of the following scientific concepts:

    a. ecology by:

        1) identifying carbon compounds as a source of food.
        2) discussing microbial degradation of oil.
        3) describing environmentally friendly products.

    b. indicators by:
        1) identifying a color change as indicative of a chemical reaction.

Background

Bioremediation

In addition to their ability to degrade cooking oils, some microorganisms can degrade petroleum-based oils. The microbial degradation of various types of oil is one aspect of the biological degradation of toxic compounds that is called bioremediation. In this process microorganisms that have some unusual metabolic pathways degrade toxic compounds, such as petroleum products. Scientists can often select microbial strains for their ability to degrade various compounds, such as crude oil. Oil spills can be “seeded” with these oil-degrading organisms along with inorganic nutrients that enhance their growth on the oil. This technique has been used on oil spills at sea as well as on those that reach the shore. The microorganisms slowly break down the long-chain hydrocarbons in the oil to carbon dioxide and water. Oil degradation is an aerobic process; therefore, air must be provided to the contaminated site to stimulate the microbial degradation of oil.

Tetrazolium Indicator

Tetrazolium is an indicator dye that is colorless in its oxidized form, but pink when it is reduced. When microorganisms metabolize carbon compounds they make waste products that serve as reducing agents, also called reductants or electron donors, that will reduce tetrazolium, turning it pink. Therefore, when bacteria metabolize a particular carbon source, they make reducing agents and the tetrazolium turns pink. In the lab today you will use this chemical reaction, the reduction of tetrazolium from its oxidized (colorless) form to its reduced (pink) form, as a measure of microbial metabolism.

Materials and Equipment (for each lab group)

4 borosilicate culture tubes (18 x 150)
4 caps (18 mm)
1 test tube rack
marking pen
6 dropping pipettes (1 ml plastic)
8 ml 0.02% tetrazolium indicator
3 ml drain cleaners (at least 3 kinds)
2-3 ml cooking oil (each group will test one kind)

Needed For Preparation:
2-3 weigh boats
balance
label tape or marking pen
flasks and tubes

Sources of Supplies

Carolina Biological Supply
2700 York Road
Burlington, NC 27215
(800) 334-5551

Description	Stock number	Quantity
18 x 150 culture tubes	K3-73-1464	case of 500
18 mm caps	K3-73-1474	100

Sigma Chemical Co.
P.O. Box 14508
St. Louis, MO 63178

Description	Stock number	Quantity
2,3,5 triphenyl tetrazolium chloride	T 8877	5 g

Preparation of Solutions

Timeline

- Prepare tetrazolium stock solution (0.2%) before the lab.
- Dilute the tetrazolium and set up the materials on the day of the lab.
- Results should take 1-5 days; if there is no change after 5 days keep tubes a few days longer.

2,3,5 triphenyl tetrazolium chloride: Make a stock solution of 0.2% tetrazolium by adding 0.1 g tetrazolium powder to 50 ml distilled water. Mix thoroughly. Store in the refrigerator. Make a fresh 0.02% tetrazolium solution the day of the lab by mixing 1 part 0.2% tetrazolium with 9 parts distilled water. Put solution in a flask or bottle with a 1 ml dropping pipette. Caution students not to use the pipette for any other solution.

Cooking oils: You can supply the oils if you don't want to have the students bring their own. Canola, olive, and generic vegetable oils seem to work well. Assign one oil per group, but give different oils to different groups.

Drain Cleaners: These products have been tested for oil degradation. We found that the bacteria in these products metabolized cooking oils but not petroleum-based motor oils.

Drain Care Build-Up Remover
Roebic K-67 Bacterial Drain and Trap Cleaner
Roebic RV and Marine Holding Tank Treatment
Roebic K-37 Septic Tank Treatment
Rid-X Septic System Treatment

Prepare drain cleaners according to manufacturer's directions.

Teacher Hints and Troubleshooting

1. Caution students that if a reagent has a pipette, do not use that pipette in any other solution.

2. If an incubator is available, the temperature should be set at 30°C to 32°C. If no incubator is available, the reactions will take longer at room temperature.

3. Two teacher demos: Streak the soil solutions and the drain cleaner solutions on nutrient agar plates to show the presence of bacteria in both types of solutions. If you want your students to do this as part of the labs, tape the dishes after they are inoculated. Do not allow students to open the dishes. Observations should be made through the lids.

4. Finger vortexing is a simple way to mix solution in a test tube. Hold the top of the tube securely in one hand and draw the index finger of the other hand toward you, gently tapping the tube. This creates a whirlpool effect inside the tube.

Student Data Sheet Answer Key

1. Hypothesis:

   Student hypotheses will vary. One example: If the drain cleaner bacteria can use (metabolize) an oil, then the tube contents will turn pink. (change colors)

2. What results would support your hypothesis? Explain.

   Students should describe the change in color as evidence of microbial metabolism.

3. Describe and explain your control.

   The two controls in this experiment that would be acceptable are: + or - oil; + or - drain cleaner.

4. Data table:

Drain cleaner name				
	Tube 1	*Tube 2*	*Tube 3*	*Control tube*
Day 0	*clear*	*clear*	*clear*	*clear*
Day 1	*pink or clear*	*pink or clear*	*pink or clear*	*clear*
Day 2	*pink or clear*	*pink or clear*	*pink or clear*	*clear*

Analysis

5. Did all drain cleaners react the same? Which ones showed the most evidence of microbial metabolism?

   Answers will vary.

6. Compare your results with classmates who used a different oil.
   Did all oils show evidence of microbial metabolism?
   Explain your answer.

   Answers will vary.

7. Why do you think some bacteria might grow better with certain oils as a food source compared to other oils?

   Presence of different enzymes or metabolic pathways allows some bacteria to use one oil but not another.

8. Find out what type of oil your family uses. Based on the results from this lab, which drain cleaner might work best if your kitchen drain became clogged? Explain your answer.

   Students should use their lab data to draw conclusions and explain their answer.

9. Do you think bacteria could degrade petroleum oil products? Would all bacteria be effective for treating oil spills?

   Yes. Some bacteria would have metabolic pathways to degrade petroleum oil, but not all bacteria (actually very few bacteria can degrade petroleum oil).

Dear Parents,

Over the next few days your sons and daughters will be doing some experiments that involve looking at drain cleaning products that use environmentally safe forms of bacteria to “eat” oils and other materials that build up in drains and septic tanks. Your child will find out what kinds of oils the microbes in these cleaners will break down.

Check with your child periodically to find out how all of this is progressing! Not only will you enjoy the time with your child, you may find a better way of cleaning your kitchen drain!

Thank you, as always, for your interest in our activities.