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EDVO-Kit

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Bioremediation Using Oil-Eating Bacteria

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

This bioremediation experiment provides students inquiry-based options to vary the parameters for determining the optimum conditions for the conversion of oil into water-soluble "natural" components.

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This experiment is designed for 10 student groups.

Experiment Components

Store all components at room temperature.

- Oil-eating microbes (powder)
- Growth media
- Petri plates
- 1N HCl
- 1M NaOH
- pH paper

Requirements

- Shaking platform or stir plate (with stir bar)
- Glassware
- Various cooking oils such as vegetable, canola, olive, peanut, etc.
- Balance

Background Information

Every year, million of gallons of oil are spilled into the environment. The biggest spill ever occurred during the 1991 Persian Gulf war when about 240 million gallons spilled from oil terminals and tankers off the coast of Saudi Arabia. The second biggest spill occurred over a ten-month period (June 1979 - February 1980) when 140 million gallons spilled at the Ixtoc I well blowout in the Gulf of Mexico near Ciudad del Carmen, Mexico.

After the Exxon Valdez accident at Bligh Reef in the spring of 1989, scientists and concerned environmentalists worked tirelessly on the beaches of Prince William Sound, Alaska to clean the 11 million gallons of spilled oil from animals, rocks, and the surrounding area. Fortunately for the environment, there were also billions of other tiny "workers" busy ridding the water and beaches of the thick, black oil. These microscopic organisms are called oleophilic bacteria, or Oil Eating Microbes (OEMs). They are bacteria that naturally use oils in the environment as their food source.

Remediation is defined as any process used to make the environment safe by absorbing, destroying, neutralizing or making harmless contaminants or decreasing them to acceptable levels. The process of using microbes to decompose hazardous substances (such as oil) to their basic, non-toxic elements, is an example of bioremediation.

This experiment will focus mostly on the use of OEMs to clean-up food oil. Oil is made up mostly of hydrocarbons, which OEMs consume. The basic process of cleaning up an oil spill involves applying a special OEM-containing solution to the spill. First, the solution breaks down the oil to molecule size, thus increasing the surface area. The increase in surface area begins the oxygenation process; this revives dormant microbes so they will begin feeding on the hydrocarbons. Nutrients in the OEM solution help these activated microbes survive.

There are three basic types of hydrocarbons: straight chains, branched chains, and 6-member rings. The OEMs break down all three of these hydrocarbons into fatty acids or carboxylic acid, which are then further broken down for energy and carbon atoms, which then are used in the citric acid cycle to generate energy. Thus, oil is broken down into basic, non-toxic elements - carbon, carbon dioxide, and water.

In order to survive, OEMs require air, water, and a source of nutrients such as oil. In order to work successfully for bioremediation, OEMs need an environment with a temperature of –2 to 60°C, and a pH of 5.5 to 10. Other factors that can inhibit the success of OEMs in bioremediation are lack of oxygen, moisture, or mineral nutrients, as well as detrimental concentrations of waste. Once these factors are corrected, OEMs can begin to do their work.

Why Bioremediation is Necessary

Petroleum is a mixture of hydrocarbons - chemicals containing the elements carbon and hydrogen. These compounds include propane, gasoline, lube oil, naphthalene (moth balls), and asphaltenes (highway blacktop). As a result of petroleum transportation and natural processes (green plants make hydrocarbons), millions of tons of these compounds enter the oceans every year. About 6 million (0.3%) of the 2 billion tons of petroleum produced each year are accidentally spilled in the oceans.

Many hydrocarbons dissolve slowly in water. Others, such as the aromatic compounds like benzene, are more soluble. These are toxic to living cells. The aromatic hydrocarbons can

Background Information

attack the fat-like membranes surrounding cells and adversely affect their normal functioning. Fortunately, bacteria and other microorganisms composing the marine flora are able to feed upon the wide variety of compounds found in petroleum. The ocean water itself aids the process by helping to transport oxygen and minerals to the microorganisms. The oil spilled in coastal areas would persist were it not for the bacteria. Other micro-organisms metabolize oil too, as do higher organisms. Whereas humans, plants, and other animals do not gain energy from ingested hydrocarbons, many species of microorganisms - bacteria, yeasts and fungi - obtain both energy and tissue-building material from petroleum. The fuel-eating bacteria, known as Pseudomonas, have evolved a taste for hydrocarbons, the major component of fossil fuels.

Through genetic engineering, scientists can enhance the ability of bacteria to metabolize petroleum. There have been attempts to develop an oil-eating "super bug." However, even without the "super bugs", the oceans have a high capacity to biodegrade petroleum. Recently, under islands in the Arctic Ocean, scientists found active hydrocarbon degradation underway even during the cold and darkness of the winter. Thus, it seems that the principal cure for oil pollution is almost everywhere in the oceans and has been with us for quite some time.

If left alone, spilled crude oil will be naturally degraded by both biological and non-biological mechanisms. So why bother using OEMs? The problem with oil is the potential short-term environmental damage. Though it is not considered a hazardous waste, crude oil coats and kills sea life and alters the surrounding beaches, rocks, trees, etc. If left to nature, oil spills will continue to plague local ecosystems indefinitely; however, OEMs degrade hydrocarbons and clean a spill to restore the environment.

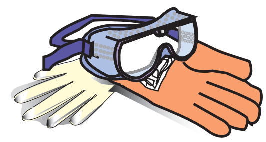
Inquiry-based Experiment Extensions for Oil Eating Microbes (OEM)

This activity is designed to foster inquiry-based research driven by students and guided by teachers to yield a meaningful hypothesis, observation(s) and conclusion(s). As in the research community, the possibilities that drive the development of pedagogy in science education is limited by the availability of reagents, equipment, time and resources required to pursue investigations.

EXPERIMENT OBJECTIVE:

This bioremediation experiment provides students inquiry-based options to vary the parameters for determining the optimum conditions for the conversion of oil to water hydrocarbons. Bacterial bioremediation is a national goal for the restoration and cleanup of contaminated land and water bodies. Oil-eating bacteria are often isolated from nature or have been modified by scientists to break down and convert oil to harmless organic compounds.

LABORATORY SAFETY



1. Gloves and goggles should be worn routinely as good laboratory practice.

2. Exercise extreme caution when working with equipment which is used in conjunction with the heating and/or melting of reagents.

3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.

4. Although the Oil Eating Microbes (OEM) used in this experiment are not considered pathogenic, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with bacteria.

5. Properly dispose materials after completing the experiment:

   A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.

   B. All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with bacteria should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:

      - Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.

      - Soak in 10% bleach solution.

      - Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.

6. Wear gloves, and at the end of the experiment, wash hands thoroughly with soap and water.

Student Experimental Procedures

CORE EXPERIMENTS

The initial experiments can be performed over the course of several weeks.

A. Set up the Oil Eating Microbes (OEM) to grow in a flask.

The number of initial culture flasks required depends on the number of student groups per class and how the experiment is designed. We recommend that you start with at least a four to five flasks, so that each flask can be tested with a different parameter (eg. pH or temperature).

- To each clean 250 ml flask, add 125 ml tap water.
- Add 1.3 g OEM powder to each flask.
- Provide adequate stirring with a stir bar plate or a shaker platform and allow the OEM cells to grow overnight at room temperature until a milky suspension is observed.

B. After a milky suspension is evident, the OEM experiment is ready to be performed with two controls.

- The Water Control Flask:
  Contains 1.3 g OEM powder grown in 125 ml water over the course of a few days as outlined in step A. **No oil is added to this flask.**

- The Oil Control Flask:
  To set up, add 125 ml tap water to a clean 250 ml flask. Add approximately 7-10 ml of food oil (vegetable oil, olive oil, peanut oil, etc.) to the tap water.

- The OEM experimental flasks:
  Contain oil-eating microbes grown in water for a few days as outlined in step A. To each of these flasks (approximately 130 ml), add 7-10 ml of food oil (vegetable oil, olive oil, peanut oil, etc.). Different groups can experiment with different types of food oil (vegetable oil, olive oil, peanut oil, etc.), and compare results with other groups among the class.

C. After the addition of the oil, allow the OEM to go to work breaking down the oil at room temperature for 2-3 days, and then proceed with your lab extension of choice as outlined in the following pages. Over the next 2-3 days, compare the experimental flasks to the control flasks and note the level of the oil in each case.

Student Experimental Procedures

LAB EXTENSIONS

Before proceeding with the lab extensions, instructor should dispense samples from flasks for each group. From each of the 2 controls and the experimental flasks (from Core Experiments), aliquot 25 ml into several smaller volume vessels (eg. beakers, baby jars, or small clear plastic food vessels) and label them accordingly. The volume in the initial flasks from Core Experiments should be at least 130 ml. Each group should have enough reagents for several experiments.

Suggestions for experiments are shown below. Students will be researching the optimum conditions of Oil Eating by Microbes. The series of lab extensions in this experiment will illustrate the response of the OEM to various environmental growth conditions that can affect cell growth.

Lab extension 1: What is the Optimum Temperature for OEM Growth?

While room temperature has been effective, what would be the effect of changing the temperature of this experiment? Try growing at 37°C (if an incubator is available) or at 4°C in a regular laboratory refrigerator. It is recommended that different temperatures be tested. Suggested temperatures are 4°C, 20°C, 37°C, and 50°C. It is important to maintain accurate temperatures.

- To alter temperature, place the aliquots (the two controls and experiments) in the refrigerator, at room temperature, and at 37°C or 50°C in an incubator.
- Allow the cells to grow and observe differences between the different samples over the course of several days.

Question: What is the optimum temperature for oil digestion among all the temperature options?

Lab Extension 2: What is the Optimum pH for OEM Growth?

This lab extension will allow students to use dilute NaOH (a base which raises pH) and dilute HCl (an acid which lowers pH) to adjust the pH of the OEM growth medium used for growing the oil-eating microbes. Suggested pHs are 5.0, 7.0, and 9.0.

Use caution when working with NaOH and HCl – wear gloves and safety goggles!!

- To the control and experimental aliquots from Core Experiment, add one or two drops of HCl to decrease the pH of the solution. Add one or two drops of NaOH to another sample to increase its pH.
- Mix the solution well, and use pH paper to check the pH of each sample. Adjust by adding additional NaOH or HCl until the desired pH is obtained.
- Allow the cells to grow over several days and observe the difference among the aliquots over the course of several days.

Question: What is the optimum pH for oil digestion among the three pH options?

Student Experimental Procedures

Student Experimental Procedures

Lab Extension 3: How Much OEM Do You Need to Digest a Fixed Amount of Oil?

- Dispense the control culture into variable volumes of microbes for the reaction (5ml, 10ml, 15ml, etc.).
- To each aliquot, add 2-3 ml of the of food oil (vegetable oil, olive oil, peanut oil, etc.).
- Allow the cells to grow and observe the difference among the aliquots over the course of several days.

Question: Which tube shows the most degradation of oil?

Lab Extensions 4: What is the effect of adding nutrients to stimulate growth of the OEM. With this option, students can make adjustments to the nutrient source in the OEM growth medium and obtain the optimum growth.

- To the control and experimental samples from Core Experiments, add a small amount (¼ or ½; teaspoon) of glucose or table sugar (sucrose).
- If available, microbe nutrients such as yeast extract can also be tested.
- Allow the cells to grow and observe the difference among the aliquots over the course of several days.

Question: Which aliquot shows the most degradation of oil?

Material Safety Data Sheets

Full-size (8.5 x 11”) pdf copy of MSDS is available at www. edvotek.com or by request.

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Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): **1 N HCl**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 03/26/11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
1MHCl CAS# 7647-01-0				

Section III - Physical/Chemical Characteristics

Boiling Point	384° F	Specific Gravity (H_2O = 1)	1.2
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	1.3	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Water soluble

Appearance and Odor: Colorless

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used): No data

Flammable Limits: LEL N.D. UEL N.D.

Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, or appropriate foam

Special Fire Fighting Procedures: Wear SCBA and protective clothing to prevent contact with skin and eyes

Unusual Fire and Explosion Hazards: Emits toxic fumes under fire conditions

Section V - Reactivity Data

Stability: Unstable ☐ Stable X — Conditions to Avoid: None

Incompatibility: Acetic Anhydride-alc. Hydrogen Cyanide, AL, Bases, Bronze, CA HCl

Hazardous Decomposition or Byproducts:

Hazardous Polymerization: May Occur ☐ Will Not Occur ☐ — Conditions to Avoid:

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Fumes cause irritation of throat, coughing/choking. Skin contact is corrosive.

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Skin irritation, inflammation, ulceration

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Wash with large amounts of water

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear protective equipment

Waste Disposal Method: Observe all Government regulations

Precautions to be Taken in Handling and Storing: Store away from incompatibles

Other Precautions: Avoid contact

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved respirator

Ventilation: Local Exhaust: Yes; Mechanical (General): No; Special: None; Other:

Protective Gloves: Chemical resistant — Eye Protection: Safety goggles

Other Protective Clothing or Equipment: Lab coat

Work/Hygienic Practices: Avoid contact - wash thoroughly after handling

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Sodium Hydroxide

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 03/26/11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Sodium Hydroxide CAS # 1310-73-2	2mg/m3	2mg/m3	No data	

Section III - Physical/Chemical Characteristics

Boiling Point	1390°C	Specific Gravity (H_2O = 1)	2.13
Vapor Pressure (mm Hg.)	20°C	Melting Point	318°C
Vapor Density (AIR = 1)	NO data	Evaporation Rate (Butyl Acetate = 1)	NO data

Solubility in Water: 10% appreciable

Appearance and Odor: White pellets, odorless

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used): NA

Flammable Limits: LEL NA UEL NA

Extinguishing Media: Use extinquishing media appropriate for surrounding fire

Special Fire Fighting Procedures: Wear protective equipment and self-contained breathing apparatus. Floof material with water

Unusual Fire and Explosion Hazards: Contact with moisture or water generate sufficient heat to ignite other materials. React with metals to produce hydrogen gas which can form explosive mixture with air.

Section V - Reactivity Data

Stability: Unstable ☐ Stable X — Conditions to Avoid: Moisture

Incompatibility: Water, strong acids, metals, combustible materials, organic materials Zinc, aluminous, peroxide, halogenated hydroca

Hazardous Decomposition or Byproducts: None identified

Hazardous Polymerization: May Occur ☐ Will Not Occur X — Conditions to Avoid:

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): None identified

Carcinogenicity: None — NTP? No data — IARC Monographs? No data — OSHA Regulation? No data

Signs and Symptoms of Exposure: Ingestion: Severe burns to mouth, throat, and stomach, nausea & vomiting Inhalation: irritation Skin/eye contact: severe irritation or burns

Medical Conditions Generally Aggravated by Exposure: None identified

Emergency First Aid Procedures: Call physician. Ingestion: Do not induce vomiting. Give water followed by vinegar, juice or egg white Inhalation: Move to fresh air. Skin/eye contact: flush with water

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Dispose of properly Wear SCBA and protective clothing. Carefully place material into clean, dry container and cover.

Waste Disposal Method: Follow all federal, state, and local laws.

Precautions to be Taken in Handling and Storing: Keep container tightly closed. Store in corrosion-proof area. Store in a dry area. Isolate from incompatible materials.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved respirator

Ventilation: Local Exhaust: Yes; Mechanical (General): Yes; Special: No; Other: None

Protective Gloves: Neoprene gloves — Eye Protection: Safety goggles

Other Protective Clothing or Equipment: Uniform, apron

Work/Hygienic Practices: Avoid contact