Microbial bioremediation of oil

Harmful chemical compounds are consistently introduced into the environment. Human activities, especially spills, waste, and pollution increase widespread contamination and lead to ecological damage. The presence and the concentration of these chemical compounds pose a threat to the biosphere, therefore decontamination of the environment is important.

Some of the chemicals that can contaminate the seas, groundwater, and soil are fortunately removed by naturally occurring microorganisms. These organisms prevent contaminants such as organic waste, heavy metals, and oil from entering the food chain.

Oil spills of crude petroleum pose serious threat to both flora and animals in the marine environment. Oils spills can be removed naturally from the environment through a process called bioremediation.

In the break down of the oil, the bacteria absorb oxygen and nutrients, then the hydrocarbons are spilt into fatty acids and further broken down to yield non-toxic by-products including metabolites, carbon dioxide, and water. Oxygen and nutrients are vital to oil-eating microbes or **OEMs** for proper degradation.

The hydrocarbons in oil are natural carbon compounds found in the environment. In the bioremediation of oil, the organisms involved are **oleophilic** (oil-loving) bacteria or *oil eating microbes* (OEMs). Oleophilic bacteria are

normally found in marine environments, but soil is also an excellent source for OEMs.

In order to observe the action of these microbes. You will simulate the process of bioremediation using OEMs to digest food or car oils under varying conditions (Light, Dark, Aerated, Sealed, Temperature). A dye indicator is used to detect active OEMs growing in culture containing oils. A colorless dye, tetrazolium indicator, turns red when bacteria are metabolically active in culture. You can observe the ideal optimal conditions in which OEMs are metabolically active in the breakdown of oil by simply observing the various concentrations of the red dye in the medium, rather than measuring the oil content.

Materials:

1. 100 ml Culture flask of bacteria from Rid-X
2. car engine oil (6 different types; 1 type/group)
3. (10) Test tubes (10ml) with push caps
4. (2)-10 ml disposable pipettes
5. 1ml pipette tips, pipettes
6. 5 ml of Peptone Nutrient solution (0.1, 1.0, and 2%) 1 per table.
7. Foil
8. Tetrazolium dye
9. Sterile Mineral oil
10. Parafilm
11. 15ml of Sterile Deionized water per group
12. Incubators at 4°C, 30°C, and 55°C.

Methods

1. Work in groups, each group receives a different type of oil.

2. Label all your test tubes with the appropriate condition. You will be testing the following conditions:

a. Light (25°C)

b. Dark (25°C)

c. 4°C, 30°C, and 55°C

d. Nutrient Solution 1 (0.1% Peptone)

e. Nutrient Solution 2 (1.0% Peptone)

f. Nutrient Solution 3 (2% Peptone)

g. Aerobic

h. Anaerobic

3. For the tube labeled "Dark" use a piece of foil paper in order to fully cover the test tube.

4. Use a 1ml pipette and add 1ml of sterile deionized water into each tube.

5. Use a 1ml pipette to transfer 1 ml of the oil into each tube.

6. Use a 1ml pipette to add 1ml of OEM culture into each tube.

7. Place 1ml of nutrient solution 1 into its respective tube. Repeat this process for the other nutrient solution concentrations.

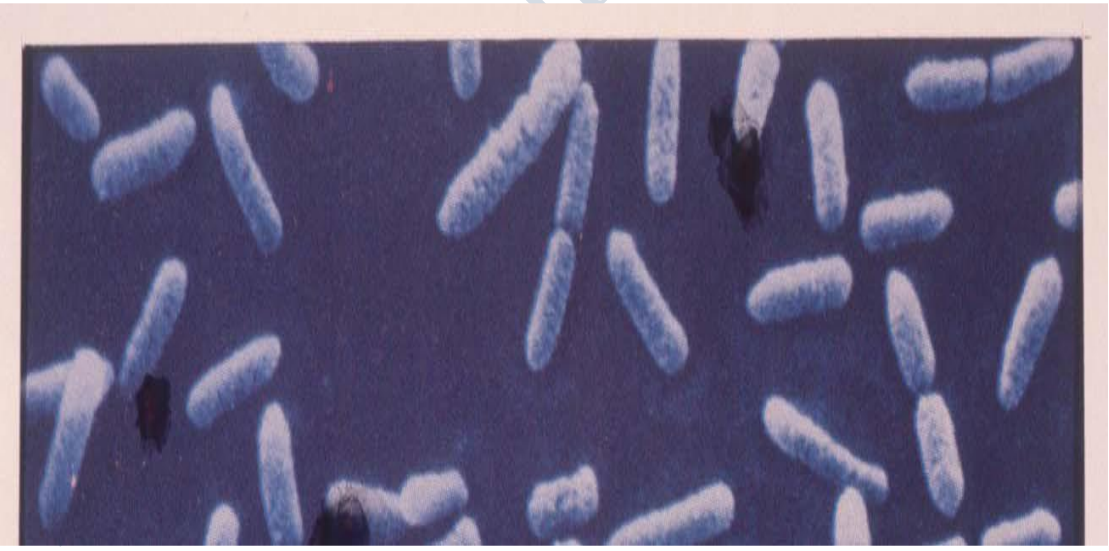
8. Add 0.1 ml of Tetrazolium dye into each tube and stir slightly.

9. Place caps over all the tubes.

10. For the anaerobic tube slowly pipette 1ml of sterile mineral oil onto the top of the liquid, cover with parafilm over and then the push cap.

11. Place each of your tubes in the correct tube racks as labeled by your TA. All other tubes unless noted are to be incubated at 30°C.

12. Next lab, observe each of your tubes and mark -, +, ++, and +++ on your data sheet according to the amount of growth present (if any) as indicated by the color of the dye.



Oil-Eating Bacteria