**“Sheen Screen” Demonstration of the Presence of**

**Oil Degrading Bacteria in the Environment**

**Introduction**

Oil spills are releases of oil (crude and refined oil products) into the environment. This happens in many locations all over the world every day. Types of oil releases vary from small amounts of automotive oil spilled on the ground to natural seeps in oil bearing geologic formations. Often, because these spills are not seen as a major problem (even though all oil spills must be dealt with according to the Federal Oil Pollution Control Act and its emergency management programs) in some instances little, if anything, might be done to address a spill if small amounts of oil are involved. Sometimes, however, large spills occur because of mechanical or human failures. Well-known examples of this include the *Exxon Valdez* spill in Prince William Sound, Alaska in 1989 and more recently the *Deepwater Horizon* catastrophe in the Gulf of Mexico in 2010.

The environmental impacts of these large spills are significant. Oil in the terrestrial or aquatic environment can affect everything from bacteria to plants to birds to whales. Spilled oil can pool in depressions on the land percolate into soil reaching underground aquifers, float on the ocean surface, wash up on beaches or remain suspended in the water column. When significant spills occur, it is important that as much of the oil as possible be removed from the environment. This is a difficult task.

One strategy that has gained more importance in fighting oil spills is the idea of **bioremediation**, the use of microorganisms or biological agents to break down or remove a substance from the environment. The use of oil degrading bacteria to reduce the amount of oil in the environment from spills is a powerful technique. These bacteria are found in the environment naturally and their use can be a very effective strategy for dealing with oil in the environment.

*Source of idea or activity:*

This activity is based on a method of demonstrating the presence of oil degrading bacteria in the environment developed by E.J. Brown and J.F. Braddock and published in the journal Applied Environmental Microbiology 56:3895-3896. Modifications to this technique were developed at the

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**Procedure for the Demonstration of the**

**Presence of Oil Degrading Bacteria in the Environment**

First Period:

1. Label the top 2 rows of the plate “Soil” and the bottom 2 rows “Onondaga”. Label the columns from left to right with “0”, “20”, “40”, “60”, “80” and “100”.

2. Use a sterile pipette to add 1.5 ml of sterile Bushnell-Hass broth to each well in the culture plate.

3. Use a sterile pipette tip to add 20µl of sterile water to each of the wells in column 1.

3. Carefully measure a 10 gram sample of soil and Onondaga Lake sediment. Add the soil or sediment to a dilution bottle with 90ml of sterile water. This gives a 1:10 dilution of the original sample.

4. Cap the bottle and shake for exactly one minute. This should put any bacteria in the sample into suspension.

5. Using a sterile pipette tip place 20µl of the soil sample suspension in the **second** well of the top row. Add 40µl of sample to the third well, 60µl to the fourth well, 80µl to the fifth well and 100µl to the sixth and final well.

7. Repeat this procedure for the second row of wells so that you have 2 replicates.

8. Inoculate the third and fourth rows with the Onondaga Lake sediment suspension using the same volumes.

9. Using a 1 ml pipette place a drop (~1/10 ml) of oil on top of each well.

10. Incubate the plate at 30ºC.

Second Period:

1. To determine whether a well shows a positive result simply look for disruption and clumping of the oil on the surface of the well. This will give the well a speckled appearance. The idea is that if even one oil degrading bacterium was in the sample placed in that well and it survived and reproduced, the resulting population of bacteria have produced a chemical that acts as a surfactant and breaks up the oil so it can be more easily digested. Wells where no bacteria are present should show no change in the oil covering the well. The chosen medium is lacking carbon so that bacteria that grow are specifically using the introduced oil as their carbon source.

**Lab Report:**

### Lab #19 20 points

Name: Date:

**Results**

Describe the appearance of the oil layer at the top of each well using simple terms such as: **intact, clumpy, dispersed, speckled, cloudy, or absent**.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample | 0 | 20 | 40 | 60 | 80 | 100 |
| Soil 1 |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |
| Onondaga  1 |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |

**Questions**

1. What was the purpose of putting Bushnell-Haas broth in the wells instead of simply using water?

2. What was responsible for the changes in appearance that you observed in the wells? If there were no changes explain why that may have happened?

3. Did one of the samples that was tested have more positive results than other? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Hypothesize possible reasons for the differences.

4. Predict what would happen if an oil spill occurred on the site where your positive samples were collected. Specifically address what would happen to the populations of bacteria present in the soil.

5. How would you propose to isolate a species of bacteria capable of degrading hydrocarbons from this experiment?