

## The Race to Remediate Crude Oil Spills

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### Background

The accidental release of carcinogenic and neurotoxic hydrocarbons from crude oil is currently a major environmental problem. The stability of these compounds enables them to persist in the environment, so their toxicity has a long-term impact on all forms of life. In 1989, the *Exxon Valdez* spilled 260 000 to 750 000 barrels of crude oil when it was grounded in Prince William Sound, Alaska [1]. Despite the extensive cleanup efforts, a study conducted by the National Oceanic and Atmospheric Administration (NOAA) determined that less than 10% of the oil had been recovered as of early 2007. What's more, 26 000 U.S. gallons of the crude oil remained in the sandy soil of the contaminated shoreline, declining at a rate of less than 4% per year and continuing to cause significant damage to the wildlife populations in the area [2]. Shoreline habitats are especially vulnerable to oil spills since contamination with these compounds can cause extensive damage to the local flora and fauna, resulting in mutations or death [3]. Various strategies, including *in situ* burning, skimming, dispersion, absorption and washing, have been used to clean up oil spills depending on the type of shoreline. However, these conventional methods come with environmental and economic costs and have only limited success since petroleum hydrocarbon compounds are difficult to remove once bound to soil components [4]. In addition, they can be intrusive and disruptive to the contaminated sites. Bioremediation, the addition of biological organisms to contaminated environments to cause an acceleration of the natural biodegradation process, has emerged as a promising option because a large percentage of oil components are biodegradable.

## **Purpose**

This experiment will try to demonstrate that the addition of hydrocarbon degrading microorganisms will enhance oil biodegradation in contaminated marine shorelines and that it is a cost effective and an environmentally benign technology.

## **Hypothesis**

An inoculum containing *Aspergillus niger* and *Pseudomonas aeruginosa*, whose growth is stimulated by the addition of a soil conditioner, is superior in bioremediation power than the sole use of either microorganism.

## **Procedure**

### **Part 1: Activation of the Microorganisms**

**1) Activation of *Pseudomonas aeruginosa*.** A small piece of freeze-dried *P. aeruginosa* was aseptically transferred to tryptic soy broth and incubated at 35°C for 48 hours.

**2) Activation of *Aspergillus niger*.** A small piece of freeze-dried *A. niger* was aseptically transferred to potato dextrose broth and incubated at room temperature for 72 hours.

### **Part 2: Preparation of the Microcosm Jars**

Four microcosms of marine shorelines, consisting of 500 g of sand, 100 ml of salt water (35% salinity) and 50 ml of crude oil were built in separate pickle jars. One jar with no inoculums was set aside and labelled as the control (Jar #1).

**1) Preparation of the Microcosm with *Pseudomonas aeruginosa*.** 100 g of treated chicken manure pellets were added to the tryptic soy broth containing *Pseudomonas aeruginosa*. 100 ml of the mixture was transferred to a pickle jar and labelled as Jar #2.

**2) Preparation of the Microcosm with *Aspergillus niger*.** 100 ml of the potato dextrose broth containing *Aspergillus niger* was added to create Jar #3.

**3) Preparation of the Microcosm with the Mixed Inoculums.** A mixture of 50 ml of *Aspergillus niger* and 50 ml of *Pseudomonas aeruginosa* was added together with 50 g of treated chicken manure to the fourth microcosm (Jar #4).

### **Part 3: Bioremediation**

All four jars were kept at 25°C for an incubation period of six weeks, and daily observations were made. The jars were aerated by being inverted 2 to 3 times each day.

### **Part 4: Extraction**

**1) Separation of the Water and Oil Emulsion.** To extract the residual crude oil from the sand, boiling hot water (100°C) was added to each microcosm. The water and oil emulsion was decanted into a separatory funnel, cooled and ultra pure hexane was added. The above step was repeated three times for each of the microcosms.

**2) Isolating the Oil From Hexane.** The combined hexane extract was washed with water and passed through Whatman #4 filter paper into a pre-weighed Erlenmeyer flask. The filter paper was washed with 10 ml of hexane, which was then added to the flask. The hexane extract was evaporated to dryness on a hot water bath, dried in a 105°C oven for one and a half hours, and then cooled in a desiccator. The procedure was repeated for each of the other microcosms.

**3) Determining the Amount of Crude Oil.** The recovered crude oil from each microcosm was weighed and the amount was compared to the control.

### **Results**

After six weeks, the crude oil levels were observed to have changed significantly in all jars except the control, where 99.97% of the crude oil was recovered. The mixture of *P. aeruginosa* and *A. niger* was found to have consumed the most oil at 52.16%, while *P. aeruginosa* and *A. niger* were each found to have consumed 32.39% and 13.21%, respectively.

## **Conclusions**

The microcosm with the mixed inoculums consumed the most oil as hypothesized, and the microcosm with only *Pseudomonas aeruginosa* was found to be the least expensive at \$130,379/ton of oil, while *A. niger* was \$1,209,122/ton. The most cost-effective was the microcosm with the mixed inoculums at \$192,858/ton. Given that current shoreline cleanup costs can reach \$294,000/ton [5], the experiment shows that the bioremediation of crude oil with different microorganisms is both a non-intrusive and cost-effective method.

However, the success of oil bioremediation depends on having the appropriate microorganisms in place under suitable environmental conditions and upon the chemical composition of the oil spilled. The rate of biodegradation depends on the type of compounds and their solubility in water: aliphatic hydrocarbons are the most readily degraded, while large, polar compounds are the least susceptible. As no single strain of bacteria has the metabolic capacity to degrade all the components found within crude oil, the experiment shows that a mixture of species of microbes should be used to obtain better results. While bioremediation is not a fast process, it is both a cost effective and an environmentally beneficial method to clean up oil spills on the moderately sensitive and most sensitive marine shorelines.

In addition, the components of the recovered crude oil are being analyzed through gas-liquid chromatography. Results concerning the specific composition of the recovered crude oil are forthcoming.

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