The Race to Remediate Crude Oil Spills
Stephanie Chan

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. Whatôs 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 35ęC 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 25ęC 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 (100ęC) 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 105ęC 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.

Acknowledgements

I would like to thank Mr. Charles Ng, senior consultant at Creation's Garden, for letting me use the equipment and reagents from his laboratory.

References

1. Bluemink, E. (2010, June 15). Size of Exxon spill remains disputed. *Anchorage Daily News*. Retrieved from http://www.adn.com/2010/06/05/1309722/size-of-exxon-spill-remains- disputed.html
2. MacAskill, E. (2007, February 2). 18 years on, Exxon Valdez oil still pours into Alaskan waters. *The Guardian.* Retrieved from http://www.guardian.co.uk/business/2007/feb/02/oil.pollution
3. P. J. J. Alvarezand, T. M. Vogel, ñSubstrate interactions of benzene, toluene, and paraxylene during microbial degradation by pure cultures and mixed culture aquifer slurries,ò Applied and Environmental Microbiology, vol. 57, no. 10, pp. 2981ï 2985, 1991.
4. S. Barathi and N. Vasudevan, ñUtilization of petroleum hydrocarbons by Pseudomonas fluorescens isolated from a petroleum-contaminated soil,ò Environment International, vol. 26, no. 5-6, pp. 413ï 416, 2001.
5. Etkin, D.S., 1999. "Estimating Cleanup Costs of Oil Spills", in *Proceedings of the 1999 International Oil Spill Conference,* pp. 1-7.

Bibliography

1. Wilson, E. (2010). Oil spill's size swells. *Chemical & Engineering News*, *88*(39), Retrieved from
2. http://pubs.acs.org/cen/news/88/i39/8839notw7.html doi: 10.1021/CEN092210132651
3. (2010, May 3). How theyŏre cleaned up. *CBC News.* Retrieved from http://www.cbc.ca/technology/story/2010/04/28/f-oil-spill-cleanup-how-its-done.html#socialcomments
4. T. M. April, J. M. Foght, and R. S. Currah, ñHydrocarbon- degrading filamentous fungi isolated from flare pit soils in northern and western Canada,ò Canadian Journal of Microbiology, vol. 46, no. 1, pp. 38ï 49, 2000.
5. Petro-Clear. (2010). *Bioremediation bacteria for hydrocarbons in soil, water, oil spills, land farming*. Retrieved from http://www.bioremediate.com/petrochemical.html
6. S. A. Adebusoye, M. O. Ilori, O. O. Amund, O. D. Teniola, and S. O. Olatope, ñMicrobial degradation of petroleum hydrocarbons in a polluted tropical stream,ò World Journal of Microbiology and Biotechnology, vol. 23, no. 8, pp. 1149ï 1159, 2007.
7. Sangeetha, R., I. Arulpandi and A. Geetha, 2011. Bacterial lipases as potential industrial biocatalysts: An overview. Res. J. Microbiol., 6: 1-24.
8. K. S. M. Rahman, T. J. Rahman, Y. Kourkoutas, I. Petsas, R. Marchant, and I. M. Banat, ñEnhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients,ò Bioresource Technology, vol. 90, no. 2, pp. 159ï 168, 2003.
9. 13. Todar, K. (2008). *Pseudomonas*. Retrieved from http://www.textbookofbacteriology.net/pseudomonas.html
10. J. G. Leahy and R. R. Colwell, ñMicrobial degradation of hydrocarbons in the environment,ò Microbiological Reviews, vol. 54, no. 3, pp. 305ï 315, 1990.
11. C. E. Zobell, ñAction of microorganisms on hydrocarbons,ò Bacteriological Reviews, vol. 10, pp. 1ï 49, 1946.
12. R. M. Atlas, ñMicrobial degradation of petroleum hydro- carbons: an environmental

perspective,ò Microbiological Reviews, vol. 45, no. 1, pp. 180ï 209, 1981.

13. R. M. Atlas, Ed., Petroleum Microbiology, Macmillion, New York, NY, USA, 1984. 18. R. M. Atlas and R. Bartha, ñHydrocabon biodegradation and oil spill bioremediation,ò
14. Advances in Microbial Ecology, vol. 12, pp. 287ï 338, 1992.
15. J. M. Foght and D. W. S. Westlake, ñBiodegradation of hydrocarbons in freshwater,ò in Oil in Freshwater: Chemistry, Biology, Countermeasure Technology, J. H. Vandermeulen and S. R. Hrudey, Eds., pp. 217ï 230, Pergamon Press, New York, NY, USA, 1987.
16. R. M. Atlas, ñEffects of temperature and crude oil com- position on petroleum biodegradation,ò Journal of Applied Microbiology, vol. 30, no. 3, pp. 396ï 403, 1975.