NRT SCIENCE AND TECHNOLOGY COMMITTEE

Fact Sheet: Bioremediation in Oil Spill Response

An information update on the use of bioremediation.

May, 2000

1. The purpose of this fact sheet is to provide on scene coordinators and other decision-makers with the latest information on evolving technologies that may be applicable for use in responding to an oil spill. Bioremediation is one technique that may be useful to remove spilled oil under certain geographic and climatic conditions. For the purpose of this effort, bioremediation is defined to include the use of nutrients to enhance the activity of indigenous organisms and/or the addition of naturally-occurring non-indigenous microorganisms. This fact sheet is an update of the NRT Science and Technology's 1991 Bioremediation fact sheet.

2. Bioremediation is a technology that offers great promise in converting the toxigenic compounds of oil to nontoxic products without further disruption to the local environment. Bioremediation is typically used as a polishing step, after conventional cleanup methods have been used. Bioremediation products considered for use during spill cleanup operations must be listed in accordance with the requirements of Subpart J of the National Contingency Plan (for further information on product listing, please consult EPA's Oil Program website at <u>www.epa.gov/oilspill</u>). Genetically engineered organisms are <u>not</u> being considered for use at this time by EPA for oil spill and are therefore not discussed in this fact sheet.

REQUIREMENTS FOR SUCCESS

3. Several factors influence the success of bioremediation, the most important being the type of bacteria present at the site, the physical and chemical characteristics of the oil, and the oil surface area. The two main approaches to oil-spill bioremediation are: (1) *bioaugmentation,* in which oil-degrading bacteria are added to supplement the existing microbial population, and (2) *biostimulation*, in which nutrients, or other growth limiting substances, are added to stimulate the growth of indigenous oil degraders.

4. Addition of oil-degrading bacteria has not been shown to have any long-term beneficial effects in shoreline cleanup operations because:

5. The size of the hydrocarbon-degrading bacterial population usually increases rapidly in response to oil contamination, and it is very difficult, if not impossible, to increase the microbial population over that which can be achieved by biostimulation alone[1-4];

6. The carrying capacity of most environments is probably determined by factors such as predation by protozoans, the oil surface area, or scouring of attached biomass by wave activity that are not affected by bioaugmentation; and

7. Added bacteria seem to compete poorly with the indigenous population.[5,6]

8.
Under the appropriate conditions, biostimulation has been shown to have beneficial effects in shoreline cleanup operations. The main challenge associated with biostimulation in oil-contaminated coastal areas or tidally influenced freshwater rivers and streams is maintaining optimal nutrient concentrations in contact with the oil.

NUTRIENT APPLICATION 9.

Effective bioremediation requires that (1) nutrients remain in contact with the oiled material, and (2) nutrient concentrations are sufficient to support the maximal growth rate of the oil-degrading bacteria throughout the cleanup operation.

10.
<u>Open Water Environments</u>. Bioremediation of open water spills is not considered to be appropriate or achievable because of the above two requirements. When nutrients are added to a floating slick, they immediately disperse into the water column, essentially diluting to background levels. At such levels rapid conversion of the hydrocarbons to biomass, CO_2, and other innocuous end products would not be readily supported.

11.
<u>Marine Environments</u>. Contamination of coastal areas by oil from offshore spills usually occurs in the intertidal zone where the washout of dissolved nutrients can be extremely rapid. In 1994 and 1995, studies were conducted on the shorelines of Delaware[7] and Maine[8] to study the rate of nutrient transport in low and high energy sandy beaches. These studies found that surface application of nutrients (including slow-release or oleophilic formulations) is ineffective on high-energy beaches because most of the nutrients are lost to dilution at high tide. However, on low

11. (continued)

energy beaches surface application of nutrients was found to be an effective and economical bioremediation strategy. Subsurface application of nutrients might be more effective on high-energy beaches but because crude oil does not penetrate deeply into most beach matrices, it is difficult to insure that the nutrients reach the oil-contaminated area near the surface.

12.
Freshwater Environments. An oil spill is most likely to have the greatest impact on wetlands or marshes. Less research has been conducted in these types of environments, so it is not yet known how well bioremediation would enhance oil removal. However, the same principles apply to this type of environment as in the marine environment: nutrients must remain in contact with the oiled material, and nutrient concentrations must be sufficient to support the maximal growth rate of the oil-degrading bacteria. There is an added complication in a wetland; oil penetration is expected to be much lower than on a porous, sandy marine beach. Below only a few centimeters of depth, the environment becomes anaerobic, and petroleum biodegradation is likely to be much slower even in the presence of an adequate supply of nitrogen and phosphorus. Technology for increasing the oxygen concentration in such an environment is still undeveloped, other than reliance on the wetland plants themselves to pump oxygen down through the root system. By the year 2000, however, data will be available from an intentional oil spill study being conducted jointly by the U.S. EPA and Fisheries and Oceans-Canada on a freshwater shoreline of the St. Lawrence River in Quebec. This study is examining bioremediation with nitrate and ammonium in the presence and absence of wetland plant species (*Scirpis americanus)*.

13.
Soil Environments. Land-farming techniques have been used extensively by petroleum companies and researchers for treating oil spills on soil. Again, the same principles apply: nutrients must remain in contact with the oiled material, and nutrient concentrations must be sufficient to support the maximal growth rate of the oil-degrading bacteria. For surface contamination, maintenance of an adequate supply of oxygen is accomplished by tilling. The maximum tilling depth is limited to about 15 to 20 inches. If the contamination zone is deeper, other types of technologies are used, such as bioventing, composting, or use of biopiles, all of which require addition of an external supply of forced air aeration.

14.
FIELD EVIDENCE FOR BIOREMEDIATION

Demonstrating the effectiveness of oil spill bioremediation technologies in the field is difficult because the experimental conditions cannot be controlled as well as is in the lab. Nevertheless, well-designed field studies can provide strong evidence for the success of a particular technology if one can convincingly show that (1) oil disappears faster in treated areas than in untreated areas and (2) biodegradation is the main reason for the increased rate of disappearance. Convincing demonstration of an increased rate of oil degradation was provided from a field study conducted during the summer of 1994 on the shoreline of Delaware Bay[9]. Although substantial hydrocarbon biodegradation occurred in the untreated plots, statistically significant differences between treated and untreated plots were observed in the biodegradation rates of certain hydrocarbon compounds.

15.
To distinguish between oil lost by physical means and oil that has been biodegraded, biodegradable constituents are normalized to a resistant biomarker compound. Hopanes often serve as this biomarker compound because they are highly resistant to biodegradation and exist in all crude oils. Normalizing to hopane automatically accounts for disappearance of oil by physical washout mechanisms. In refined oils that have no hopanes biodegradation can be confirmed by normalizing to a highly substituted 4-ring PAH or by examining the relative rates of disappearance of alkanes and PAH homologs.

16.
It is important to note that some bioremediation products contain surfactants and emulsifiers that change the appearance and mobility of the oil. These processes should be distinguished from true biodegradation.

OTHER RESEARCH

17.
Research is ongoing to evaluate bioremediation and phytoremediation (plant-assisted enhancement of oil biodegradation) for their applicability to clean up oil spills contaminating salt marshes and freshwater wetlands. By December of 2000, EPA is planning to produce a draft guidance document detailing the use of bioremediation for sandy marine beaches and freshwater wetlands. EPA is also studying the biodegradability of non-petroleum oils (vegetable oils and animal fats) and their impacts on the environment during biodegradation. Reports will be available some time in 2000 and 2001.

CONCLUSION

18.
In conclusion, bioremediation is a proven alternative treatment tool that can be used in certain oil-contaminated environments. Typically, it is used as a polishing step after conventional mechanical cleanup options have been applied. It is a relatively slow process, requiring weeks to months to effect cleanup. If done properly, it can be very cost-effective, although an in-depth economic analysis has not been conducted to date.

18. (continued)

One of the advantages to using bioremediation products is that the toxic hydrocarbon compounds are destroyed rather than simply moved to another environment. The biggest challenge facing the responder is maintaining the proper conditions for maximal biodegradation to take place, i.e., maintaining sufficient nitrogen and phosphorus concentrations in the pore water at all times. Based on field experiments and solid evidence from the literature it has been shown that addition of exogenous cultures of microorganisms will not enhance the process more than simple nutrient addition and that bioremediation is less effective on high energy shorelines.

The NRT S&T Committee technical contact for bioremediation issues is Dr. Albert D. Venosa of the Environmental Protection Agency. He can be reached at venosa.albert@epa.gov.

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OIL SPILL EATER INTERNATIONAL (OSEI, CORP.) EVALUATION OF THE NRT SCIENCE AND TECHNOLOGY COMMITTEE FACT SHEET: MAY 20, 2000

Paragraph 1. Is a Statement of the Fact Sheet's Purpose.

It is unfortunate that Dr. Venosa chose to only use nutrients for the tests performed for this Fact Sheet. We agree - nutrients alone will not work - and Dr. Venosa proves this fact in his Fact Sheet. Dr. Venosa keeps pushing nutrients which are very limited as to the spill conditions in which they may be used effectively; as Dr. Venosa points out.

Paragraph 2.

Explains that Bioremediation offers significant promise in converting the toxigenic compounds of oil to non-toxic products without further disruption to the environment. Again, Dr. Al Venosa (EPA Laboratory) keeps pushing nutrients but then proves they do not work. How does this help the On-Scene Coordinators?

Paragraph 3. Requirements for Success.

They describe Biostimulation as nutrients or other growth-limiting substances, but they fail to mention or test those Bioremediation Products that utilize nutrients all the other constituents to emulate Mother Nature.

Paragraphs 4 through 7.

We agree with the EPA Fact Sheet. For eleven years we have stated that using indigenous bacteria to clean up oil spills works faster and more effective than adding bacterial product.

Paragraph 8.

They explain that under the appropriate conditions, biostimulation has been shown to have beneficial effects on shoreline treatments. This statement needs to be qualified as nutrients only (which Dr. Venosa keeps pursuing) are limited as to the conditions in which they may be used.

OIL SPILL EATER II is not limited the way nutrients are. In fact, in a letter dated April 20, 2000, Mr. Venosa agreed to the fact that when OSE II is applied to oil, it adheres to the oil. This means wave action will not wash away OSE II and dilute it. This means OSE II can be used in active inter-tidal zones, as well as open ocean settings and fresh water fast moving rivers.

Paragraph 9. Nutrient Application.

OSEI, Corp. concurs with this paragraph since OSE II does exactly what Dr. Venosa states is necessary for "effective Bioremediation." OSE II (1) adheres to the oil and (2) supplies the concentration of all nutrients necessary for effective Bioremediation.

Paragraph 10. Open Water Environments.

They state that Bioremediation of open waters is not considered appropriate or achievable. What Dr. Venosa is really stating is that what nutrients alone are limited as to where they can be used. This is not true for OIL SPILL EATER II (OSE II), since it molecularly adheres to the oil and Dr. Venosa has so stated and knows that OSE II does.

How does Dr. Venosa explain and ignore the fact that for one and one/half years OSE II has been successfully and effectively used at the Navy Fuel Farm in San Diego, CA for oil spills on U.S. Navigable Waters, with the Coast Guard and the State of California present? The oil is cleaned up and with no adverse effects to the San Diego Bay ECO System.

Furthermore, Dr. Venosa has been fully appraised of these facts. He obviously is choosing to ignore the fact that at least one Bioremediation Product does work effectively on water. Dr. Venosa needs to change this statement in the Fact Sheet since he has misled the NRT', the RRT's and particularly the OSC's.

Paragraph 11. Marine environments.

OSEI, Corp. concurs with their comments, but they are only applicable to nutrients, - not OIL SPILL EATER II.

Paragraph 12. Fresh Water.

OSEI, CORP. agrees with the EPA - nutrients have limited capabilities; however, OSE II breaks up the oil in small droplets, OSE II "floats" the oil (hydraulic lifting) and OSE II molecularly adheres to the oil. OSE II will only minimally increase the BOD (See Enclosure #1 - BOD statement by Dr. Theron Miller). If the BOD becomes a problem in an enclosed environment, simply aerating the oil-covered water with pumps, will allow rapid Biodegradation of the oil and eliminate the BOD problem.

Paragraph 13. Soil Environments

Again, nutrients (fertilizers) do not adhere to the oil and, how many nutrients do you apply? OSE II has been solving this problem for 11 years. We have been cleaning up soil that is contaminated with hydrocarbons very effectively and at a tremendous savings in cost.

Paragraph 14. Field Evidence for Bioremediation.

The Fact Sheet states that it is difficult to demonstrate Bioremediation in the field vs. the lab. OSE II has cleaned up contaminated soils all over the U.S., Alaska, Korea and Japan.

Using Dr.Venosa's nutrients, it is impossible to demonstrate for the reasons mentioned previously, i.e., nutrients do not adhere to the oil; how much product (nutrients) do you use; and Dr.Venosa's nutrients do not contain all the nutrients necessary for the complete bacterial growth. OSE II provides all the nutrients needed and can tell the user exactly how much OSE II to apply.

Paragraph 15.

OSEI, Corp. has proven that OSE II does, in fact, biodegrade oil. Dr. Brown of the University of Alaska, ran a scientifically valid test to prove that OSE II does biodegrade alkanes and PAH's. Dr. Venosa has this test and is fully aware that OSE II works whereas his nutrients will not. (See Enclosure 2, a copy of Dr. Brown's Test.).

Paragraph 16. BIOREMEDIATION - WHAT IT REALLY IS!

OIL SPILL EATER II
CHEMICAL PROCESS

Once OSE II is applied to a hydrocarbon spill, the enzymes and other product constituents start emulsification and solubalization of the hydrocarbon substrate. Emulsification and solubalization generally take from a few minutes up to a few hours for heavy-end hydrocarons, once OSE II is applied, with a Temperarture of 40 degrees F. or greater. Once solubilization is completed, the hydrocarbon substrate is less toxic (and the hazard of a fire is diminished) the enhanced, naturally occurring bacteria will have a higher affinity for the solubilized, hydrocarbon substrate.

NOTE: There is no hydraulic loading with the use of OSE II and therefore treated hydrocarbons are not pushed into the lower depths of the water column. During these reactions, OSE II offers up a complete nutrient system to promote the rapid growth or colonization of naturally occurring, indigenous bacteria.

OSE II is also formulated so that once application to the hydrocarbon substrate occurs, molecular adhesion takes place. This prevents OSE II from being removed from the hydrocarbons easily. The above reaction forms the substrate complex.

Once the outer molecular walls of the hydrocarbon substrate complex have been weakened or broken, then this allows bacteria better access to the hydrocarbon substrate. The nutrients in OSE II's product matracies (readily available nitrogen, phosphorous, carbon and vitamins), rapidly populates naturally occurring bacteria. There are certain product constituents to enhance various hydrocarbon- degrading bacteria specifically. The naturally enhanced hydrocarbon degrading bacteria rapidly populate until product nutrients are depleted, at which time they readily convert to the only food source left - the weakened or broken hydrocarbon substrate. The transition state complex is when the enhanced naturally occurring hydrocarbon degrading bacteria start converting hydocarbons to C02 and water.

The enhanced naturally occurring hydrocarbon degrading bacteria convert the solubalized hydrocarbons to C02 and water which is the end point or the Bioremediation of the hydrocarbon substrate. Any OSE II product components left are 100% biodegradable and will be used up naturally.

Dr. Venosa explains that having surfactants and emulsifiers preclude a product from being true Bioremediation. This is somewhat a misrepresentation of the facts, because in Mother Nature - when bacteria become proximal to a spill they release surfactants and enzymes to help breakdown hydrocarbon structures (detoxify) so the bacteria can utilize the spilled contaminant as a food source. OSE II has the same nutrients that Mr. Venosa pushes, plus we have all the constituents that occur in Mother Nature to speed up Bioremediation. To call Dr. Venosa's limited, and incomplete nutrients true Bioremediation over complete products that supply all of the constituents up front that are required by Mother Nature renders this fact sheet as nonfactual itself.

Paragraph 17.

OSE II is ideally suited for all applications - fresh or salt water - open water - beaches and marshes.

Chapter 18. Conclusions.

Mechanical cleanups (the method of choice) allows 80% of the oil to sink into the water. OSE II, on the other hand, FLOATS the oil, and rapidly detoxifies the oil, thereby protecting the ECO System and by rapidly Biodegrading the oil..

There are cost comparisons available and Dr. Venosa has this data. The Navy at the San Diego Fuel Farm has reduced their mechanical cleanup cost for oil spills on water from $90.00/spilled gallon to $12.00/spilled gallon and only $1.00 of the $12.00 cost is for OSE II.

CONCLUSION - BY: OSEI, CORP.

OSEI, Corp.'s OIL SPILL EATER II, solves all the problems spelled out in this Fact Sheet associated with Dr. Venosa's attempt to use and evaluate only nutrients.

OIL SPILL EATER II is successfully and effectively used on oil spills on soil and U.S. Navigable Waters.

OIL SPILL EATER II (OSE II) should be pre-approved by all RRT's for use on oil spills.

By: Steven R. Pedigo
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