

## **NRT FACT SHEET: BIOREMEDIATION IN OIL SPILL RESPONSE**

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### **SUMMARY**

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.

### **BACKGROUND**

Many compounds in crude oil are environmentally benign, but significant fractions are toxic or mutagenic. The latter are the ones we are most interested in removing or destroying in an oil spill. Bioremediation is a technology that offers great promise in converting the toxic compounds to nontoxic products without further disruption to the local environment.

When microorganisms break down petroleum hydrocarbons, the first step usually is addition of a hydroxyl group to the end of an alkane chain or onto an unsaturated ring of a polycyclic aromatic hydrocarbon (PAH), forming an alcohol. Progressive oxidation to an aldehyde and then a carboxylic acid leads to chain length reduction and eventually to production of carbon dioxide, water, and biomass. In the case of the PAH, ring fission takes place, again leading eventually to mineralization. As oxygen is added to hydrocarbons, the compounds become more polar and thus more water soluble. These compounds are usually more easily biodegradable and

thus less toxic. Although the more polar compounds are more likely to enter the water column as biodegradation ensues, they are unlikely to cause environmental damage or toxic effects to nearby biota. Furthermore, the amount of dilution available from the tidal waters is so great that the amounts of benign polar constituents entering the food chain are likely to be negligible. Thus, the effect of biochemical end products from the easily metabolizable compounds in oil will be insignificant in the environment.

### **REQUIREMENTS FOR SUCCESS**

Since the contaminants of concern in crude oil are readily biodegradable under appropriate conditions, the success of oil-spill bioremediation depends on our ability to establish those conditions in the contaminated environment. The most important requirement is that bacteria with appropriate metabolic capabilities must be present. If they are, their rates of growth and hydrocarbon biodegradation can be maximized by ensuring that adequate concentrations of nutrients and oxygen are present and that the pH is between about 6 and 9<sup>1</sup>. The physical and chemical characteristics of the oil are also important determinants of bioremediation success. Heavy crude oils that contain large amounts of resin and asphaltene compounds are less amenable to bioremediation than are light- or medium-weight crude oils that are rich in aliphatic components. Finally, the oil surface area is extremely important because growth of oil degraders occurs almost exclusively at the oil-water interface<sup>1</sup>.

Obviously, some of these factors can be manipulated more easily than others. For example, nothing can be done about the chemical composition of the oil, and no adequate engineering approaches are currently available for providing oxygen to oil-contaminated surficial sediments in the intertidal zone. Therefore, 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 co-substrates are added to stimulate the growth of indigenous oil degraders. Since oil-degrading bacteria usually grow at the expense of one or more components of crude oil, and these organisms are ubiquitous<sup>2-4</sup>, there is usually no reason to add hydrocarbon degraders unless the indigenous bacteria are incapable of degrading one or more important contaminants. 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<sup>5-8</sup>. 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 added bacteria seem to compete poorly with the indigenous population<sup>9</sup>. Therefore, it is unlikely that they will persist in a contaminated beach even when they are added in high numbers. As a result, bioaugmentation has never been shown to have any long-term beneficial effects in shoreline cleanup operations.

Biostimulation involves the addition of rate-limiting nutrients to accelerate biodegradation by indigenous microorganisms. When an oil spill occurs, it results in a huge influx of carbon into the impacted environment. Carbon is the basic structural component of living matter, and in order for the indigenous microorganisms to be able to convert this carbon into more biomass, they need significantly more nitrogen and phosphorus than is normally present in the environment. Both of these elements are essential ingredients of protein and nucleic acids of living organisms. 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**

Effective bioremediation requires nutrients to remain in contact with the oiled material, and the concentrations should be sufficient to support the maximal growth rate of the oil-degrading bacteria throughout the cleanup operation.

Marine Environments. With respect to the marine environment, 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. Oleophilic and slow-release formulations have been developed to maintain nutrients in contact with the oil, but most of these rely on dissolution of the nutrients into the aqueous phase before they can be used by hydrocarbon degraders. Therefore, design of effective oil bioremediation strategies and nutrient delivery systems requires an understanding of the transport of dissolved nutrients in the intertidal zone.

Transport through the porous matrix of a marine beach is driven by a combination of tides, waves, and flow of freshwater from coastal aquifers. Tidal influences cause the groundwater elevation in the beach and the resulting hydraulic gradients to fluctuate rapidly. Wave activity affects groundwater flow through two main mechanisms. First, when waves run up the beach face ahead of the tide, some of the water percolates vertically through the sand above the water line and flows horizontally when it reaches the water table. Waves can also affect groundwater movement in the submerged areas of beaches by a pumping mechanism that is driven by differences in head between wave crests and troughs.

In 1994 and later in 1995, tracer studies were conducted on the shorelines of Delaware<sup>11</sup> and Maine<sup>12</sup> to study the rate of nutrient transport in low and high energy, sandy beaches. The Delaware work showed that the

rate of tracer washout from the bioremediation zone (i.e., upper 25 cm below the beach surface) was more rapid when tracer was applied at spring tide than at neap tide, but the physical path taken by the tracer plume moved vertically into the beach subsurface and horizontally through the beach in a seaward direction. Vertical transport was driven by waves, whereas horizontal transport was driven by tides. The Maine work suggested that surface application of nutrients would be ineffective on high-energy beaches because most of the nutrients will be lost to dilution at high tide. On low energy beaches, however, this is an effective and economical bioremediation strategy. Nutrients that are released from slow-release or oleophilic formulations will probably behave similarly to the dissolved lithium tracer that was used in the study. Thus, they will not be effective on high-energy beaches unless the release rate is high enough to achieve adequate nutrient concentrations while the tide is out. Subsurface application of nutrients might be more effective on high-energy beaches. Since crude oil does not penetrate deeply into most beach matrices, however, nutrients must be present near the beach surface to effectively stimulate bioremediation. Since nutrients move downward and seaward during transport through the intertidal zone of sandy beaches, nutrient application strategies that rely on subsurface introduction must provide some mechanism for insuring that the nutrients reach the oil-contaminated area near the surface.

Freshwater Environments. With respect to freshwater shorelines, an oil spill is most likely to have the greatest impact on wetlands or marshes rather than a wide shoreline zone like a marine intertidal zone. Less research has been conducted in these types of environments, so it is not yet known how well bioremediation would enhance oil removal. 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*). However, the same principles apply to this type of environment as a marine environment, namely, that nutrients must be maintained in contact with the degrading populations for a sufficient period of time to effect the enhanced treatment. There is an added complication in a wetland, however. 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 to the rhizosphere through the root system.

Soil Environments. Land-farming techniques for treating oil spills on soil have been used extensively for years by petroleum companies and researchers. Again, the same principles apply: maintenance of an adequate supply of limiting nutrients and electron acceptors (nitrogen, phosphorus, and oxygen) in contact with the degrading populations throughout the entire treatment period. 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, however. If the contamination zone is deeper, other types of technologies would have to be used, such as bioventing, composting, or use of biopiles, all of which require addition of an external supply of forced air aeration.

## **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 possible 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<sup>13</sup>. Although substantial hydrocarbon biodegradation occurred in the untreated plots, statistically significant differences between treated and untreated plots were observed in the biodegradation rates of total alkane and total aromatic hydrocarbons. First order rate constants for disappearance of individual hopane-normalized alkanes and PAHs were computed, and the patterns of loss were typical of biodegradation. Significant differences were not observed between plots treated with nutrients alone and plots treated with nutrients and an indigenous inoculum of oil degraders from the site. The high rate of oil biodegradation that was observed in the untreated plots was attributed to the relatively high background nitrogen concentrations that were measured at the site.

#### OTHER RESEARCH

Continuing 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. Data will be available in the year 2000 for the freshwater wetland study and 2001 for the salt marsh. 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.

#### CONCLUSION

In conclusion, bioremediation is a proven alternative treatment tool that can be used to treat certain aerobic 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. It has the advantage 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 (~5-10 mg N/L). Based on solid evidence from the literature, it appears that addition of exogenous cultures of microorganisms will not enhance the process more than simple nutrient addition. Bioremediation is not considered a primary response tool, although it could be so used if the spilled oil does not exist as free product and if the area is remote enough not to require immediate cleanup to satisfy a tourism industry. If the affected environment is a high energy shoreline, bioremediation will be less likely effective than on a lower energy shoreline. Application of dry granular fertilizer to the impact zone is probably the most cost-effective way to control nutrient concentrations.

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