

Preamble to the Proposed RRT VI Bioremediation Position Paper  
RRT VI Science & Technology Committee  
January 2001

The attached document is an adaptation of the National Response Team Science and Technology Committee's May, 2000 "Fact Sheet: Bioremediation in Oil Spill Response." The intended audience is on-scene coordinators and others familiar with oil spill response methods and technologies.

While agreeing with the content of the original NRT document, the RRT VI Science and Technology Committee believes that modifications were required to provide clarification and emphasis on issues of special interest to this region. Specifically, issues regarding the use of bioremediation in open-water and marine environments, recent field studies, and guidance in RRT pre-approval.

No material was deleted from the original NRT document. The RRT VI modifications appear in italics for the reader's convenience.

This paper should be viewed as a living document which must be periodically revisited to account for new developments.

RRT VI Science & Technology Committee

Brian Cain (USFWS)

Donald Davis (LOSCO)

James Hanifen (LDWLF)

Charles Henry (NOAA)

Jeff Lewellin (TNRCC)

Robert D. Martin, Jr. (TGLO) - Committee Chairman

# ***RRT 6 Position Paper on Bioremediation***

(Adopted January 24-25, 2001)

*Adapted from the NRT Science and Technology Committee's*

## **Fact Sheet: Bioremediation in Oil Spill Response**

**An information update on the use of bioremediation.**

May, 2000

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.

Bioremediation is a technology that offers great promise in converting the toxic 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 [www.epa.gov/oilspill](http://www.epa.gov/oilspill)). Genetically engineered organisms are not being considered for use at this time by EPA for oil spill and are therefore not discussed in this fact sheet.

### **REQUIREMENTS FOR SUCCESS**

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.

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

- 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>1-4</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>5,6</sup>

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**

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.

Open Water Environments. 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 will immediately disperse into the water column, essentially diluting to levels close to background. At such levels, rapid conversion of the hydrocarbons to biomass, CO<sub>2</sub>, and other innocuous end products would not be supported readily. *In addition, the process of biodegradation is too slow to prevent the vast majority of the oil from reaching the shoreline.*

Marine Environments. 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<sup>7</sup> and Maine<sup>8</sup> 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 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.

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*).

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.

## 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 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>9</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 certain hydrocarbon compounds.

*Another series of experiments comparing bioaugmentation and biostimulation in both the laboratory and the field was conducted in a wetland research site along the San Jacinto River (a brackish water environment) near Houston, TX. Over a three-year period, natural attenuation, as well as biostimulation and bioaugmentation were investigated. The natural attenuation field study concluded that there was substantial biodegradation of selected petroleum contaminants over time<sup>10,11,12</sup>. Results from the biostimulation field study indicated that the biostimulation treatments accelerated the biodegradation process, as compared to the control treatment<sup>12,13</sup>. For the bioaugmentation investigations, a lab study indicated enhanced biodegradation for three out of 13 bioaugmentation products tested as compared to the naturally occurring microorganisms<sup>14</sup>. The field component of this study, however, found that there were no statistical differences in the biodegradation rates of the commercial products as compared to the naturally occurring microbes<sup>15,16</sup>.*

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. *Appropriate sampling and analysis is important because bioremediation products that contain surfactants or emulsifiers can cause a sudden change in the spilled oil's appearance. The casual observer can mistake these changes for proof of successful bioremediation.*

## OTHER RESEARCH

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.

## **EPA guidelines for Bioremediation of Marine Shorelines and Freshwater Wetlands**

## CONCLUSION

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. *Unlike some response technologies, such as dispersants or in-situ burning, bioremediation is not constrained to very narrow windows of opportunity (e.g., hours) that require Regional Response Team pre-approval in order to be effective.* 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.

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](mailto:venosa.albert@epa.gov).

## REFERENCES

1. Jobson, A.M., M. McLaughlin, F.D. Cook, and D.W.S. Westlake. 1974. *Appl. Microbiol.* 27:166-171.
2. Westlake, D.W.S., A.M. Jobson, and F.D. Cook. *Canad. J. Microbiol.* 24:245-260.
3. Lee, K. and E.M. Levy. 1987. Proc. 1987 International Oil Spill Conference, American Petroleum Institute, Washington, D.C.
4. Lee, K., G.H. Tremblay, J. Gauthier, S.E. Cobanli, and M. Griffin. 1997. Bioaugmentation and biostimulation: A paradox between laboratory and field results. pp. 697-705. In Proceedings, 1997 International Oil Spill Conference. American Petroleum Institute, Washington, DC.
5. Tagger, S., A. Bianchi, M. Juillard, J. LePetit, and B. Roux. 1983. Effect of microbial seeding of crude oil in seawater in a model system. *Mar. Biol.* 78: 13-20.
6. Lee, K. and E.M. Levy. 1989. Enhancement of the natural biodegradation of condensate and crude oil on beaches of Atlantic Canada. pp. 479-486. In Proceedings, 1989 Oil Spill Conference. American Petroleum Institute, Washington, DC.
7. Wrenn, B.A., M.T. Suidan, K.L. Strohmeier, B.L. Eberhart, G.J. Wilson, and A.D. Venosa. 1997. "Nutrient transport during bioremediation of contaminated beaches: evaluation with lithium as a conservative tracer." *Wat. Res.* 31(3):515-524.
8. Wrenn, B.A., M. C. Boufadel, M.T. Suidan, and A.D. Venosa. 1997. "Nutrient transport during bioremediation of crude oil contaminated beaches." In: *In Situ and On-Site Bioremediation: Volume 4*, pp. 267-272. Battelle Memorial Institute, Columbus, OH.
9. Venosa, A.D., M.T. Suidan, B.A. Wrenn, K.L. Strohmeier, J. R. Haines, B.L. Eberhart, D. King, and E. Holder. 1996. "Bioremediation of an experimental oil spill on the shoreline of Delaware Bay." *Environmental. Sci. and Technol.* 30(5):1764-1775.
10. Mills M. and Bonner J. 1996. *San Jacinto River oil spill: wetland bioremediation research project. In Proceedings of the Nineteenth Arctic and Marine Oilspill Program (AMOP) Technical Seminar. Environment Canada. Ottawa, Ontario, pp.647-666.*
11. Harris B. C., Bonner J.S. and Autenrieth R.L. 1999. *Nutrient dynamics in marsh sediments contaminated by an oil spill following a flood. Environmental Technology, 20:795-810.*
12. Mueller D., Bonner J., Autenrieth R., Lee K. and Doe K. 1999. *The toxicity of oil-contaminated sediments during bioremediation of a wetland. In Proceedings of the 1999 International Oil Spill Conference. American Petroleum Institute, Washington, DC, pp. 180.*
13. Mills M., Bonner J., Simon M., McDonald T. and Autenrieth R. 1997. *Bioremediation of a controlled oil release in a wetland. In Proceedings of the Twentieth Arctic and Marine Oilspill Program (AMOP) Technical Seminar. Environment Canada. Ottawa, Ontario, pp.609-616.*
14. Aldrett S., Bonner J., Mills M., Autenrieth R., and Stephens F. 1997. *Microbial degradation of crude oil in marine environments tested in a flask experiment. Wat. Res.* 31(11):2840-2848.
15. Simon M., Bonner J., McDonald T., and Autenrieth R. 1999. *Bioaugmentation for the enhanced bioremediation of petroleum in a wetland. Polycyclic Aromatic Compounds. 14 & 15:231-239.*
16. Townsend R.T., Bonner J. and Autherieth R. *Microbial dynamics during bioremediation of a crude oil-contaminated coastal wetland. Bioremediation Journal. (accepted in March 2000).*