SUMMARY OF THE LITERATURE ON THE USE OF COMMERCIAL BIOREMEDIATION AGENTS FOR CLEANUP OF OIL-CONTAMINATED ENVIRONMENTS

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ABSTRACT

The objective of this document is to conduct a comprehensive review of the use of commercial bioremediation products treating oil spills in all environments. Literature assessed includes peer-reviewed articles, company reports, government reports, and reports by cleanup contractors engaged in responses to oil spills. The scope of this review is in the general context of estuarine environments. The review will be useful for oil spill responders (e.g., on-scene coordinators and response contractors) to better understand the feasibility of bioremediation technology and as an aid in selecting bioremediation products. This state-of-science review on the efficacy of bioremediation products is conducted using different approaches and presented accordingly as follows.

The overall conclusions reached by this review are as follows. First, according to the peer-reviewed literature, bioaugmentation appears to have little benefit for the treatment of spilled oil in an open environment. Microbial addition has not been shown to work better than nutrient addition alone in many field trials. However, case studies provided by vendors seem to suggest that application of bioaugmentation products could still have some potential in the treatment of specific oil components, isolated spills in confined areas, or certain environments where oil-degrading microorganisms are deficient. Unfortunately, the evidence for such a conclusion is not strong and in most, if not all, cases are scientifically deficient.

Biostimulation has been proven to be a promising tool to treat certain aerobic oil-contaminated shorelines. In general, commercial oleophilic nutrient products have not shown clear advantages over common agricultural fertilizers in stimulating oil biodegradation. Effects of nutrients are also highly site-specific.

The extreme uncertainty associated with the efficacy of bioremediation agents is due in large part to the poorly designed field tests that have been conducted to demonstrate efficacy. Much of the reported literature either lacked proper controls and quality assurance, or the data were incorrectly analyzed. If there is any hope for advancement of commercial bioremediation, experiments based on sound scientific principles are needed.

INTRODUCTION AND BACKGROUND

Bioremediation has emerged as a promising technology, particularly as a secondary treatment option for oil cleanup. Bioremediation has been defined as "the act of adding materials to contaminated environments to cause an acceleration of the natural biodegradation processes" (OTA, 1991). This technology is based on the premise that a large percentage of oil components are readily biodegradable in nature (Atlas, 1984, 1981; Prince, 1993). Bioremediation has several potential advantages over conventional technologies, such as being less costly, less intrusive to the contaminated site, and more environmentally benign in terms of its end products.

The success of oil spill bioremediation depends on one's ability to establish and maintain conditions that favor enhanced oil biodegradation rates in the contaminated environment. Numerous scientific review articles have covered various factors that influence the rate of oil biodegradation (Zobell 1946; Atlas, 1981 & 1984; Atlas and Bartha, 1992; NAS, 1985; Focht and Westlake, 1987; Leahy and Colwell, 1990). There are two main approaches to oil spill bioremediation: *Bioaugmentation*, in which known oil-degrading bacteria are added to supplement the existing microbial population, and *Biostimulation*, in which the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth-limiting co-substrates.

Disclaimer

The information in this document was extracted from a more comprehensive report by Zhu *et al*. (2004). The original report was subjected to the Agency's peer and administrative reviews and approved for publication as an EPA document. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use.

Objectives and Scope

The objective of this document is to conduct a thorough assessment of bioremediation products by a comprehensive review of the actual use of bioremediation in real world cases. Literature assessed includes peer-reviewed articles, company reports, government reports, and actual reports by cleanup contractors engaged in responses to spills in inland, estuarine, and marine environments. This review will be useful for oil spill responders (e.g., on-scene coordinators and response contractors) to better understand the feasibility of bioremediation technology and as an aid in selecting bioremediation products.

As mentioned earlier, only a limited number of scientific, peer-reviewed journal articles are available on the performance of bioremediation products for oil spill cleanups. However, there are many reports pertaining to the use of bioremediation products in the non-peer reviewed or "gray" literature. A thorough search for the "gray" literature is an important part of this project. The in-depth review of these non-peer reviewed reports will fill the "information gap" and provide a better picture in regard to the present and potential of the use of bioremediation products as a viable option for oil spill cleanups.

It should be noted that all the reports collected are evaluated comprehensively for their scientific merit, and only those judged appropriate and scientifically sound are earmarked for inclusion in this document. The scope of this review is in the general context of estuarine environments. However, marine shorelines, terrestrial environments, freshwaters, and wetlands are frequent candidates for bioremediation of spilled oil, and these ecosystems are also included in the review for completeness.

ASSESSMENT OF BIOREMEDIATION PRODUCTS IN THE FIELD: PEER-REVIEWED LITERATURE

Field studies can provide the most convincing demonstration of the effectiveness of oil bioremediation since laboratory studies are not always able to account for numerous real world conditions such as spatial heterogeneity, biological interactions, and mass transfer limitations. Swannell *et al.* (1996) conducted the most extensive literature review available on field evaluations of oil bioremediation in marine environments. Venosa (1998) presented an in-depth critical review of research studies emphasizing extensive inadequacies in the experimental design and control of published field tests. However, none of the existing reviews has focused on the field performance of commercial bioremediation agents. They did not distinguish bioremediation due to addition of commercial products and bioremediation due to application of common agricultural fertilizers/nutrient solutions or non-commercial microbial strains.

The U.S. EPA has defined Bioremediation agents as "microbiological cultures, enzyme additives, or nutrient additives that significantly increase the rate of biodegradation to mitigate the effects of the discharge" (Nichols, 2001). To have bioremediation products used properly, the U.S. EPA has compiled a list of bioremediation agents (Nichols, 2001; USEPA, 2002) as part of the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule, which is required by the Clean Water Act. At the time of this writing, 14 bioremediation agents were listed on the NCP Schedule. The Schedule is intended for use by Federal On-Scene Coordinators (FOSCs), Regional Response Teams (RRT), and other oil spill responders as an aid in determining the most appropriate products to use in various spill scenarios.

Compared to laboratory investigations, few tests have been carried out to evaluate the effectiveness of bioremediation products in the field because such trials are both difficult and expensive to conduct. One of the most difficult tasks in field studies is the proper evaluation of oil biodegradation. Oil contaminated sites are often highly heterogeneous, where oil concentrations can vary greatly within a small area. Physical and chemical weathering may also significantly affect the composition and concentration of oil contamination. Consequently, variability associated with field studies can be so high as to preclude or interfere with one's ability to discern significant treatment differences. Nevertheless, the efficacy of bioremediation in the field can be verified through well-designed monitoring programs and proper data interpretation.

Evidence for the effectiveness of oil bioremediation should include: (1) faster disappearance of oil in treated areas than in untreated areas, and (2) a demonstration that biodegradation was the main reason for the increased rate of oil disappearance. In recent years, non-biodegradable or slowly biodegradable components in oil - often called biomarkers - have been used successfully to distinguish between biodegradation and the physical or chemical loss of oil and to mitigate the high variability associated with field studies (Bragg *et al*., 1994; Venosa *et al*., 1996; Lee *et al*., 1997). Detailed procedures to properly evaluate oil bioremediation can be found in *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands* (Zhu *et al*., 2001). All these principles will be used as the basis to evaluate the technical merit of the literature reviewed in this document.

Application of Bioaugmentation Products

The rationale for this approach is that indigenous microbial populations may not be capable of degrading the wide range of potential substrates present in complex mixtures such as petroleum (Leahy and Colwell, 1990) or that they may be in a stressed state as a result of the recent exposure to the spill.

Venosa *et al*. (1992) conducted a field test in Prince William Sound following the *Exxon Valdez* spill to investigate the effectiveness of two commercial microbial products vis-à-vis natural attenuation and nutrient addition alone. This field trial failed to demonstrate enhanced oil biodegradation by these products. No biostimulation occurred in the nutrient control plots either. There were no significant differences between any of the treatment and control plots during the 27-day trial period.

A few field trials did claim success in demonstrating the effectiveness of oil bioaugmentation, such as using Alpha BioSea™ (Alpha Environmental, Inc.) to treat the Angolan Palanca crude oil spilled from *Mega Borg* off Texas coast (Mauro and Wynne, 1990; Swannell *et al*., 1996) and using TerraZyme™ (Oppenheimer Biotechnology) in enhancing biodegradation of a heavy oil spilled from *Nakhodka* in Japan (Tsutsumi *et al*., 2000). However, the success of these studies was based on either visual observation (i.e. the *Mega Borg* study) or digital photographic image analysis (i.e., the *Nakhodka* study). No comprehensive monitoring program was used to verify the oil was indeed removed through enhanced biodegradation.

All these peer-reviewed journal articles show that even though the addition of microorganisms may be able to enhance oil biodegradation in the laboratory, the effectiveness of bioaugmentation has not been convincingly demonstrated in the field. Actually, most field studies indicated that bioaugmentation is not effective in enhancing oil biodegradation in inland, estuarine, and marine environments. It appears that in most environments, indigenous oil-degrading microorganisms are more than sufficient to carry out oil biodegradation if nutrient levels and other adverse environmental conditions do not limit them.

Application of Biostimulation Products

In most shoreline ecosystems that have been heavily contaminated with hydrocarbons, nutrients are likely the limiting factors in oil biodegradation. The one exception is wetlands. If oil has penetrated wetland or marsh sediment to any appreciable extent, the impact zone is anoxic or anaerobic, and oxygen limitation will be the predominant mechanism precluding effective treatment.

Commonly used water-soluble nutrient products include mineral nutrient salts (e.g. KNO_3, $NaNO_3$, NH_4NO_3, K_2HPO_4,

$MgNH_4PO_4$), and many commercial inorganic fertilizers. They are usually applied in the field through the spraying of nutrient solutions or spreading of dry granules. This approach has been effective in enhancing oil biodegradation in many field trials (Swannell *et al*., 1996; Venosa *et al*., 1996).

Field studies conducted in wetland environments showed that biostimulation was ineffective in treating certain oil-contaminated salt marshes or freshwater wetlands due to oxygen limitation (Garcia-Blanco *et al*., 2001; Shin *et al*., 1999; Venosa et al., 2002). In 1999 and 2000, a field study was conducted on the shoreline of the St. Lawrence River (Garcia-Blanco *et al.*, 2001; Venosa *et al.*, 2002). The authors concluded that nutrient amendment of an oil-contaminated freshwater wetland *where significant penetration of oil has taken place into the sediment* has limited potential for enhanced cleanup of the contamination.

All these results suggest that the success of biostimulation is case specific, depending on oil properties, the nature of the nutrient products and the characteristics of the contaminated environments. When oxygen is not a limiting factor, one of keys for the success of oil biostimulation is to maintain an optimal nutrient level in the interstitial pore water.

Use of slow release fertilizers is one of the approaches used to overcome washout problems and provide continuous sources of nutrients to oil contaminated areas. Slow release fertilizers are also readily available nutrient products normally in solid forms that consist of either relatively insoluble nutrients or water-soluble nutrients coated with hydrophobic materials such as paraffin or vegetable oils. Slow release fertilizers have shown some promise in oil bioremediation studies and applications. For example, following the *Exxon Valdez* accident, a slow-release granular fertilizer, Customblen (Sierra Chemical Co.), was chosen as one of the bioremediation agents to apply over 120 km of the oil-contaminated shorelines during 1989 and 1990. This fertilizer consists of vegetable oil coated calcium phosphate, ammonium phosphate, and ammonium nitrate (N:P:K ratio 28-8-0). The results showed that Customblen performed well on some of the shorelines of Prince William Sound (Atlas, 1995; Pritchard et al., 1992; Swannell et al., 1996).

Compared to microbial products, very few nutrient additives have been developed and marketed specifically as commercial bioremediation agents for oil spill cleanup. Due to the limitations of common fertilizers (e.g., being rapidly washed out due to tide and wave action), several organic nutrient products, such as oleophilic nutrient products, have recently been evaluated and marketed as bioremediation agents. Field evaluation results for some of these products have been available in the peer-reviewed literature.

Inipol EAP22 (Societe, CECA S.A., France) is currently listed on the NCP Product Schedule as a nutrient additive. Following the *Exxon Valdez* spill, Inipol EAP22 was chosen as one of the nutrient products to use in the cleanup, and approximately 50,000 kg of nitrogen and 5,000 kg of phosphorus were applied over 120 km of the oil-contaminated shorelines during 1989 and 1990. Inipol EAP22 was selected also because it was the only commercially available bioremediation agent with large production capacity at the time other than common agricultural fertilizers (Pritchard *et al*., 1992).

Based on several field studies on the effectiveness of Inipol EAP22, Sveum *et al.* (1994) indicated that this oleophilic fertilizer appeared to be more effective than water-soluble fertilizers when the spilled oil resided in the intertidal zone. But they have no advantages in enhancing oil biodegradation in the supratidal zone where water transport is limited.

Other studies on the effect of similar organic fertilizers derived from natural products also yielded mixed results. In summary, the effectiveness of these various types of nutrient formulations will depend on the characteristics of the contaminated environment and of the formulations themselves. Slow-release fertilizers may be an ideal nutrient source if the nutrient release rates are well controlled. Water-soluble fertilizers are likely more cost-effective in low-energy and fine-grained shorelines where water transport is limited. Oleophilic fertilizers may be more suitable for use in high-energy and coarse-grained beaches or less accessible rocky outcroppings. Peer-reviewed literature on the use of bioremediation products has clearly indicated that biostimulation, if used properly, could be a cost-effective treatment tool for cleaning certain oil-contaminated environments.

ASSESSMENT OF OIL BIOREMEDIATION PRODUCTS: NON-PEER REVIEWED LITERATURE

Only limited field studies and applications of bioremediation products have been reported in the peer-reviewed literature. In an attempt to better document the potential and understand the scope of the actual use of bioremediation products, a thorough search of non-peer-reviewed and 'gray' literature pertaining to the use of bioremediation agents in response to oil spills in inland, estuarine, and marine environments was conducted. A comprehensive review of this information is presented in the full version of this paper.

Government Agency Reports

Many government agencies have been involved in various aspects of oil spill cleanups. At the federal level, oil spill response planning is coordinated through the U.S. National Response Team (NRT, www.nrt.org). This is an interagency group made up of 16 federal agencies and co-chaired by the EPA and the U.S. Coast Guard (USCG), each with responsibilities and expertise in various aspects of spill response.

Several field studies or applications on the use of commercial bioaugmentation agents have been published in government agency reports with mixed results. Mearns (1991) reported on a bioaugmentation field test of an oiled marsh in an estuary environment of upper Galveston Bay, Texas. The spill occurred in August 1990, when a collision occurred between three Apex Barges and the Greek tanker Shinoussa. Four plots were used in selected areas of Marrow Marsh: two treated with a commercial bioaugmentation product (Alpha BioSea™, Alpha Environmental, Inc.), and two left untreated as controls. Oil constituents were determined using GC/MS and the extent of biodegradation was measured by the decline in the n-C18/phytane ratio in this study. Mearns *et al*. (1993) later summarized some lessons learned from this experiment, which included that bioremediation is not a rapid response tool, experimental design should meet basic statistical requirements, and more comprehensive monitoring is needed to demonstrate the efficacy of treatment.

Several government agency reports of field experience with evaluation and use of commercial nutrient products are available. Included among them are several articles that cover bioremediation experience following the *Exxon Valdez* oil spill (Bragg *et al*., 1992; Pritchard *et al*., 1991; Venosa *et al*., 1990). Field studies on the use of nutrient products have produced mixed results. For a detailed description of proper protocol for oil bioremediation field studies and evaluation, readers may refer to EPA's *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands* (Zhu *et al*., 2001).

In a manual on treating oil spills in tundra environments published by Alaska Department of Environmental Conservation, Athey et al. (2001) compiled dozens of case studies on the experience of oil spill cleanup in Alaska. Among these cases, five involved the use of bioremedial approaches, mostly biostimulation with agricultural fertilizers Vendor's Reports

To better understand the scope and potential of the use of bioremediation products, efforts were made to collect information from vendors of bioremediation agents and their service providers.

Information collection

In February 2002, 70 vendors of bioremediation agents that are listed either on the NCP Schedule or on *20th International Oil Spill Control Directory* (Oil Spill Intelligence Report, 2000) were contacted through emails or letters in regard to their interest in participating in our case study review. A total of eight vendors of bioremediation products were willing to participate in this investigation and submitted at least some the information requested. It should be noted that these cases do not necessarily reflect all cleanup efforts of each company. They are selected based on relevancy to the scope of this report, sufficient information provided, and scientific merit. One product is reviewed here.

CASE STUDIES: REVIEW OF VENDOR REPORTS

The amount and quality of the information submitted by vendors was highly variable. Case study information mostly ranged from a few sentences to one to two pages, although there were also a few detailed technical reports of up to 50 pages. It is impossible to give a comprehensive review of each case based on this limited information. Therefore, a summary of important findings and general critique on the technical merit of all these reports are presented here.

Bioremediation products have been applied to clean up petroleum hydrocarbon contamination in various ecosystems and under a wide range of environmental conditions. Their applications include *in-situ* remediation of hydrocarbon contaminated marine shorelines, soil environments, surface water, groundwater, and bilge water, and *ex-situ* treatment of hydrocarbon contaminated soil (*e.g.*, using a land treatment unit) and water (*e.g.*, in a bioreactor).

A major limitation of these vendor case studies is that due to the confounding of different effects, it is impossible to determine whether the claimed enhanced oil biodegradation, if true, resulted mainly from the addition of microbial cultures, nutrients, enzymes, oxygen, or any combination of above. The technical merit of these company reports was generally not sound in terms of providing strong or even suggesting moderate scientific evidence for demonstrating the effectiveness of bioremediation products.

SUMMARY AND FINDINGS

Bioremediation is a process that attempts to accelerate natural biodegradation processes. The success of oil spill bioremediation depends on our ability to optimize various physical, chemical, and biological conditions in the contaminated environment. Bioremediation products have been applied to clean up petroleum hydrocarbon contamination in various ecosystems and under a wide range of environmental conditions. Their applications include *in-situ* remediation of hydrocarbon contaminated marine shorelines, soil environments, surface water, groundwater, and water, and *ex-situ* treatment of hydrocarbon contaminated soil (e.g., use of land treatment units or other types of reactor systems such as compost piles, biopiles, slurry reactors, etc.) and water (e.g., in a bioreactor). Bioremediation technology is typically used as a secondary polishing step after conventional mechanical cleanup options have been applied to remove free oil product. For a more detailed discussion of conclusions from this review, the reader is referred to the original paper Zhu, Venosa, and Suidan.

- According to the peer-reviewed literature, bioaugmentation appears to have little benefit for the treatment of spilled oil in an open environment. Microbial addition has not been shown to work better than nutrient addition alone in many field trials.
- Biostimulation has been proven to be a promising tool to treat certain aerobic oil-contaminated marine shorelines. One of the key factors for the success of oil biostimulation is to maintain an optimal nutrient level in the interstitial pore water.
- Different nutrient products have shown variable effectiveness, depending on oil properties, the nature of the nutrient products, and the characteristics of the contaminated environments.
- Bioremedial approaches may have a role in treating hydrocarbon contamination for non-point sources. Limited available information appears to suggest that application of bioremediation agents could show promise for the treatment of hydrocarbon contamination in stormwater, especially used in conjunction with other stormwater countermeasures, such as wet detention ponds.
- The extreme uncertainty associated with the efficacy of bioremediation agents is due in large part to the poorly designed field tests that have been conducted to demonstrate efficacy. Much of the reported literature lacked proper controls and treatment randomization and replication, or the data were incorrectly analyzed.

If there is any hope for advancement of commercial bioremediation, field experiments based on sound scientific principles are needed.

BIOGRAPHY

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Dr. Venosa holds a B.S. degree in Microbiology and M.S. and Ph.D.degrees in Environmental Science from the University of Cincinnati. He has headed up EPA's oil spill research program for 17 years and has published over 100 peer-reviewed publications. He led 4 field studies on oil spill bioremediation and is now active in chemical countermeasure research (dispersants and surface washing agents).

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