EPA/600/R-04/075
July 2004

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

by

[1]Xueqing Zhu, [2]Albert D. Venosa, and [1]Makram T. Suidan,

[1]University of Cincinnati
Cincinnati, OH 45221

[2]U.S. Environmental Protection Agency
National Risk Management Research Laboratory
Cincinnati, OH 45268

EPA Contract No. 68-C-00-159
Task Order No. 19

Task Order Manager

Albert D. Venosa
Land Remediation and Pollution Control Division
National Risk Management Research Laboratory
Cincinnati, OH 45268

National Risk Management Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Disclaimer

The information in this document has been funded by the United States Environmental Protection Agency (U.S. EPA) under Task Order No. 19 of Contract No. 68-C-00-159 to the University of Cincinnati. It has been subjected to the Agency's peer and administrative reviews and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

Lawrence W. Reiter, Acting Director
National Risk Management Research Laboratory

Executive Summary

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. 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. 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. Section 1 provides an overall introduction of the background and the scope of this review. Section 2 presents an in-depth review of field tests of bioremediation products based on the scientific literature, which includes peer-reviewed journal articles, books, and major conference proceedings. Section 3 evaluates oil bioremediation products based on the non-peer reviewed literature articles gathered, such as government agency reports and vendor/service provider reports. Finally, Section 4 gives the conclusions and recommendations based on the reviewed information.

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 is scientifically deficient.

Biostimulation has been proven to be a promising tool to treat certain aerobic oil-contaminated shorelines. One of the key factors for the success of oil biostimulation is to maintain an optimal nutrient level in the interstitial pore water. 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. For example, the availability of oxygen rather than nutrients is often the limiting factor in wetland environments, where addition of nutrient products has not been successful in enhancing oil biodegradation.

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. Unfortunately, due to the extreme resource intensiveness of field studies, the benefit accruing to testing one bioremediation agent is only applicable to the one product being tested. Testing products in the field is not within the purview of the federal government unless such a test has the potential of advancing science in terms of general microbiological and engineering principles.

TABLE OF CONTENTS

1 Introduction and Background

It is estimated that between 1.7 and 8.8 million metric tons of oil are released into the world's water every year (NAS, 1985), of which more than 90% is directly related to human activities including deliberate waste disposal. Marine oil spills, particularly large-scale spill accidents, have received great attention due to their catastrophic damage to the environment. For example, the spill of 37,000 metric tons (11 million gallons) of North Slope crude oil into Prince William Sound, Alaska, from the *Exxon Valdez* in 1989 led to the mortality of thousands of seabirds and marine mammals, a significant reduction in population of many intertidal and subtidal organisms, and many long term environmental impacts (Spies *et al*., 1996). Minor oil spills and oil contamination from non-point source discharges (e.g., urban runoff and boat bilge) are no less threats to public health and the environment, although they have received much less attention in the past. According to recent *National Water Quality Inventory* reports, non-point source pollution remains the Nation's largest source of water quality problems (U.S. EPA, 1996&2000). It is the main reason that approximately 40 % of surveyed rivers, lakes, and estuaries are not clean enough to meet basic uses such as fishing or swimming.

Conventional oil spill countermeasures include various physical, chemical, and biological methods. Commonly used physical methods include booming and skimming, manual removal (wiping), mechanical removal, water flushing, sediment relocation, and tilling. Physical containment and recovery of bulk or free oil is the primary response option of choice in the United States for the cleanup of oil spills in marine and freshwater shoreline environments. Chemical methods, particularly dispersants, have been routinely used in many countries as a response option. However, chemical methods have not been extensively used in the United States due to the disagreement about their effectiveness and the concerns of their toxicity and long-term environmental effects (U.S. EPA, 1999). With the recent development of less toxic chemical dispersants, the potential for their applications may increase.

Although conventional methods, such as physical removal, often are the first response option, they rarely achieve complete cleanup of oil spills. According to the Office of Technology Assessment (OTA, 1990), current mechanical methods typically recover no more than 10-15 percent of the oil after a major spill, although significantly higher recoveries have been achieved, depending on the environment. Bioremediation is beginning to emerge 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). One important requirement is the presence of microorganisms with the appropriate metabolic capabilities. If these microorganisms are present, then optimal rates of growth and hydrocarbon biodegradation can be sustained by ensuring that adequate concentrations of nutrients and oxygen are present and that the pH is between 6 and 9. The physical and chemical characteristics of the oil and oil surface area are also important determinants of bioremediation success. 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.

Although extensive research has been conducted on oil bioremediation during the last decade, the effectiveness of this technology has only rarely been convincingly demonstrated, and in the case of commercial bioremediation products, the literature is virtually completely lacking in supportive evidence of success. Most existing studies have concentrated on evaluating the factors affecting oil bioremediation or testing favored products and methods through laboratory studies (Mearns, 1997). Only limited numbers of pilot-scale and field trials, which may provide the most convincing demonstrations of this technology, have been reported in the peer-reviewed literature (Prince 1993, Swannell *et al*, 1996, Venosa *et al*., 1996 and 2002). The scope of current understanding of oil bioremediation is also limited because the emphasis of most of these field studies and reviews has been on the evaluation of bioremediation technology for dealing with large-scale oil spills on marine shorelines. To help oil spill responders in the selection and application of bioremediation products, there is an immediate need to gather and evaluate information about the field performance of commercial bioremediation products, especially for dealing with low-level petroleum hydrocarbon contamination.

To better understand the potential effectiveness of bioremediation technology, Public Law 105-457 entitled "Estuaries and Clean Waters Act of 2000" (the Act) was enacted, which states specifically that "the Administrator of the Environmental Protection Agency (EPA) shall begin a two-year study on the efficacy of bioremediation products." The Act mandated that "the study shall evaluate and assess bioremediation technology (a) on low-level petroleum hydrocarbon contamination from recreational boat bilges, (b) on low-level petroleum hydrocarbon contamination from storm water discharges, (c) on non-point source petroleum hydrocarbon discharges, and (d) as a first response tool for petroleum hydrocarbon spills." This report is a part of EPA's efforts to address the Congressional mandate under the Act by extensive review of literature where bioremediation products have been used for oil spill cleanup.

1.1 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. 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.

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. Various government agencies [e.g., U.S. EPA, U.S. Coast Guard, U.S. Navy, U.S. Army, Department of Energy (DOE), the National Oceanic and Atmospheric Administration (NOAA)] have involved field investigation of bioremedial approaches for treating petroleum hydrocarbon contamination. More than 170 companies around the world are listed in *Oil Spill Intelligence Report* (2000), which offer either bioremediation products or bioremediation services. It is reasonable to assume that many field trials or applications conducted by government agencies, vendors, and responders have been documented but are not readily available to the public for various reasons. 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. If a report is deemed invalid due to technical deficiencies or insufficient information, it will also be mentioned but explanations given to why it was not an integral part of our final recommendation.

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.

1.2 Organization of the Document

This state-of-science review on the efficacy of bioremediation products is conducted using three different approaches and, accordingly, presented in the following major Sections. Section 2 presents an in-depth review of field tests of bioremediation products based on scientific literature, which includes peer-reviewed journal articles, books, and major conference proceedings (e.g., *International Oil Spill Conference* and *International Bioremediation Symposium*). Section 3 evaluates oil bioremediation products based on all the non-peer reviewed literature articles gathered, such as government agency reports and vendor/service provider reports. A discussion is also presented in Section 3 in regard to the potential of using bioremediation products in the areas of non-point source and stormwater runoff countermeasures and for treating bilge oil from boats, ships, cutters, and other watercraft. Finally, Chapter 4 gives the conclusions and recommendations based on all the information reviewed throughout this document.

2 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 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. Other reviews are also available (Prince, 1993; Leahy and Colwell, 1990). However, none of 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. This chapter will present a comprehensive review with the emphasis on the efficacy of commercial bioremediation products in the field by reviewing latest peer reviewed articles, as well as summarizing major points identified in the previous reviews. Non-commercial products or common agricultural fertilizers may also be covered only for the purpose of comparison.

2.1 Bioremediation Products and Evaluation

2.1.1 Bioremediation agents

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). Bioremediation agents are also classified as bioaugmentation agents and biostimulation agents based on the two main approaches to oil spill bioremediation. Numerous bioremediation products have been proposed and promoted by their vendors, especially during early 1990s, when bioremediation was popularized as "the ultimate solution" to oil spills (Hoff, 1993). The U.S. EPA is often inundated with salespeople wanting to have EPA endorse their products. 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, the Oil Pollution Act of 1990, and the National Contingency Plan. The Schedule is intended for use by Federal On-Screen 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.

At the time of this writing, 15 bioremediation agents were listed on the NCP Schedule as shown in Table 2.1. This list has been modified recently, and the number has been reduced to nine. A product can be placed on the Schedule only if all the required data have been submitted and when its safety and effectiveness have been demonstrated under the conditions of a test protocol developed by EPA (NETAC, 1993a; Nichols, 2001). However, the listing of a product on the Schedule does not mean that the product is approved or certified for use on an oil spill. At present, the only efficacy requirement for being listed is to pass the Bioremediation 28-Day Effectiveness Test. The test protocol uses laboratory shake flasks to compare the degradation of artificially-weathered crude oil in natural seawater with and without a bioremediation product. This test alone cannot demonstrate that a product will be effective in the field. Studies have shown that bioremediation products may be effective in the laboratory but significantly less so in the field (Lee *et al*., 1997, Mearns, 1997; Venosa *et al*., 1992 and 1996). This is because laboratory studies cannot always simulate complicated real world conditions such as spatial heterogeneity, biological interactions, climatic effects, and nutrient mass transport limitations. Therefore, field studies and applications are the ultimate tests or the most convincing demonstration of the effectiveness of bioremediation products.

Table 2.1 Bioremediation agents in NCP product schedule (Adapted from USEPA, 2002)

Name or Trademark	Product Type	Manufacture
BET BIOPETRO	MC	BioEnviro Tech, Tomball, TX
BILGEPRO	NA	International Environmental Products, LLC, Conshohocken, PA
INIPOL EAP 22	NA	Societe, CECA S.A., France
LAND AND SEA RESTORATION	NA	Land and Sea Restoration LLC, San Antonio, TX
MICRO-BLAZE	MC	Verde Environmental, Inc., Houston, TX
OIL SPILL EATER II	NA/EA	Oil Spill Eater International, Corporation Dallas, TX
OPPENHEIMER FORMULA	MC	Oppenheimer Biotechnology, Inc., Austin, TX
PRISTINE SEA II	MC	Marine Systems, Baton Rouge, LA
STEP ONE	MC	B & S Research, Inc., Embarrass, MN
SYSTEM E.T. 20	MC	Quantum Environmental Technologies, Inc. (QET), La Jolla, CA
VB591™WATER, VB997™SOIL, AND BINUTRIX	NA	BioNutraTech, Inc., Houston, TX
WMI-2000	MC	WMI International, Inc., Houston, TX

Abbreviations of product type:
MC -- Microbial Culture
EA -- Enzyme Additive
NA -- Nutrient Additive

2.1.2 Assessing oil bioremediation in the field

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. To obtain such evidence, one has to be careful in selecting proper oil analysis procedures as well as in interpreting the data. Oil analysis methods can be generally classified into two categories: nonspecific methods to measure total petroleum hydrocarbons (TPHs), and specific methods using various chromatographic techniques to quantify target oil constituents. Total petroleum hydrocarbons (TPH) techniques have been widely accepted methods to rapidly quantify the oil due to their simplicity and low costs. However, these methods are severely affected by the spatial heterogeneity and, more importantly, they are much less able to distinguish between abiotic and biotic losses. The reason for this is that conventional TPH analysis is confounded by the presence of plant lipids and other biogenic compounds that interfere with interpretation of the analysis.

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). This approach estimates the extent of biodegradation by using GC/MS techniques and evaluating the ratios of target hydrocarbon concentrations relative to the concentration of these recalcitrant biomarkers, such as hopanes and steranes and to a lesser extent alkyl-substituted 4-ring PAHs such as C_3-chrysene. Studies have shown that normalizing target oil constituents to biomarkers mitigates the spatial variability of oil contamination when compared to other mass balance approaches and allows biodegradation to be monitored effectively by reducing the number of samples required (Douglas *et al.*, 1994).

To ensure that monitored results reflect reality in a highly heterogeneous environment, it is also critical that a bioremediation sampling plan be designed according to valid statistical principles that include the principles of randomization, replication, and the use of proper controls. For example, to minimize bias, a random sampling plan should be used to evaluate treatment effects and their variance within the bioremediation zone. Efforts should also be made to ensure that an adequate number of independent samples are taken to reach a given accuracy and confidence. A proper control or untreated set aside area is also critical to demonstrate the true impact of a treatment. 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.

2.2 Application of Bioaugmentation Products

Since the 1970s, bioaugmentation, or the addition of oil-degrading microorganisms to supplement the indigenous populations, has been proposed as an alternate strategy for the bioremediation of oil contaminated environments. 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. Other conditions under which bioaugmentation may be considered are when the indigenous hydrocarbon-degrading population is low, the speed of decontamination is the primary factor, and when seeding may reduce the lag period to start the bioremediation process (Forsyth *et al*., 1995). For this approach to be successful in the field, the seed microorganisms must be able to degrade most petroleum components, maintain genetic stability and viability during storage, survive in foreign and hostile environments, effectively compete with indigenous microorganisms, and move through the pores of the sediment to the contaminants (Atlas, 1977; Goldstein *et al*., 1985).

Methods involving the addition of selected oil-degrading microorganisms into spilled oil have been patented and marketed since early 1970s (Azarowick, 1973; Linn, 1971; and Mohan *et al*., 1975). However, before the *Exxon Valdez* spill in 1989, little information on the performance of commercial bioaugmentation products was available in the peer-reviewed literature. Atlas and Bartha (1973) conducted one of the first laboratory tests on the effectiveness of commercial mixed bacterial cultures. Two commercial petroleum-degrading bacterial inocula, Ekolo-Gest (also marketed as Petrobac, National Chem. Corp.) and DBC bacteria (Gerald Bauer Corp.), were tested using shake flasks to compare the degradation of Sweden crude oil. The study found that none of the commercial mixtures was superior to the indigenous microorganisms in coastal marine waters.

One of first field trials on oil bioremediation using a microbial product in a marine environment was reported by Lee and Levy (1987). The study involved seeding a mixed culture of marine oil-degrading bacteria (strains of *Pseudomonas aeruginosa*, *Pseudomonas stutzeri,* and *Bacillus subtilis* grown on bran) in a Scotian Shelf Condensate (SSC) contaminated sandy beach. The extent of biodegradation was measured by the decline in the n-C_{17}/pristane ratio in this study. The results showed that the n-C_{17}/pristane ratio in the seeded plots did decrease slightly. However, due to high inter-and intra-plot variability, no significant difference in the rate of oil loss was observed among the treatments. This study also observed that the number of oil-degrading bacteria did not increase until 10 to 15 days after the addition of oil. However, the addition of the microbial product did not reduce this lag period, suggesting that the toxic volatile components in the oil, which evaporated mostly during the first week, was the main cause of the lag period.

Since the application of nutrient amendments for the cleanup of the *Exxon Valdez* spill in 1989, bioremediation has received increased attention, and several field tests and applications of bioaugmentation have been reported. 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. These products were selected based on a previous laboratory study (Venosa *et al.*, 1991). 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. However, the site where the project took place (Disk Island) was characterized as having highly weathered (degraded) oil and very calm waters, so dissolved oxygen may have been limiting, thus precluding effective biodegradation by any means.

One approach in overcoming the competition problem was proposed by Rosenberg *et al.* (1992). They developed a product that combined a polymerized ureaformaldehyde fertilizer, which they called F-1, with a selected oil-degrading culture capable of using this fertilizer as a nitrogen source. Thus, the culture had a selective advantage over the indigenous population unable to utilize F-1 as nutrient source. A field trial conducted at an Israeli beach showed that this approach seemed to be successful in enhancing oil biodegradation. However, conclusions were confounded by the lack of adequate controls in the study (Swannell *et al.*, 1996; Venosa, 1998).

To evaluate the effectiveness of two commercial bioaugmentation products in an estuarine environment, a field trial was carried out in a Texas coastal wetland by a research group from Texas A&M University (Simon *et al.*, 1999; Townsend *et al.*, 1999). The two products were selected based on a previous laboratory efficacy test, in which four out of twelve products showed an enhancement of oil biodegradation with significantly higher degradation rates of alkanes and aromatics when compared to a nutrient control (Aldrett *et al.*, 1997). The 21-plot site, named San Jacinto Wetland Research Facility (SJWRF) has been used for a series of studies on oil spills and their countermeasures. In this study, four treatment strategies were examined: an oiled control, biostimulation with inorganic nutrient addition (diammonium phosphate) , and commercial bioaugmentation with 2 different products. Arabian medium crude oil was selected in this test and the 21 plots each measuring 5 x 5 m were arranged in a balanced, incomplete block experimental design. Oil constituents were determined using gas chromatography/mass spectrometry (GC/MS) and were normalized to 17α(H), 21β(H)-hopane to reduce the effects of sample heterogeneity and physical losses. The results showed that the addition of microbial products could not significantly enhance oil biodegradation rates. No differences were observed between treatments when comparing the first order biodegradation rate coefficients for the total target saturates, total target aromatics, and individual hydrocarbon target analytes. The authors also pointed out that one of the products (BP8) "did show consistently higher biodegradation rates, though the rates were not significantly different from the control." Because this microbial product was applied with vendor supplied inorganic nutrients (Townsend *et al.*, 1999), it is difficult to conclude whether the "consistently but insignificantly" higher rates resulted from the additions of the microbial components or the nutrient components. The fact that neither addition of bioaugmentation agents nor application of inorganic nutrients significantly enhanced oil biodegradation suggested that other factors, such as oxygen, could have been limiting oil degradation in that environment.

Studies comparing the performance of bioaugmentation and biostimulation have suggested that nutrient addition alone had a greater effect on oil biodegradation than did the addition of microbial products when oxygen supply was not limited (Jobson *et al.*, 1974; Lee *et* *al*., 1997; Venosa *et al.*, 1996). This is probably because the hydrocarbon-degrading population is rarely a limiting factor as compared to the nutrients since the size of the hydrocarbon-degrading bacterial population usually increases rapidly in response to oil contamination. One of the first comprehensive field tests evaluating various bioremediation approaches to enhance oil biodegradation was carried out in a soil environment in northwest area of Canada in early 1970s (Jobson et al., 1974). A randomized block design was used to examine the effects of four treatments (control, inorganic fertilizer application, ; addition of a microbial culture alone , and combined fertilizer and microbial culture addition) over a 308-day time period. The microbial culture was grown in the laboratory and consisted of several genera of oil-degrading bacteria (*Flavobacterium* and *Cytophoga* sp., *Pseudomonas* sp., *Xanthomonas* sp., *Alcaligenes* sp., and *Arthrobacter* sp.). The study showed that the nutrient application resulted in a significant stimulation of bacterial numbers and in the degradation rate of n-alkane components of the crude oil. The application of the microbial agent, however, resulted in only a slightly enhanced degradation rate of n-alkane components of chain lengths C20 to C25.

A field study conducted on a sandy beach in Delaware also showed that addition of a microbial inoculum did not enhance oil biodegradation more than addition of inorganic nutrients alone (Venosa et al., 1996). A randomized block design was used in this study to assess the effects of three treatments: a no-nutrient control (natural attenuation), addition of water-soluble nutrients, and addition of water-soluble nutrients supplemented with a natural microbial inoculum from the site. No significant differences were observed between plots treated with nutrients alone and plots treated with nutrients and the indigenous inoculum, suggesting that supplementation of the natural population with indigenous cultures from the same site still did not result in further enhancement over simple nutrient addition on marine shorelines. The authors also indicated that this conclusion could be extended to include exogenous microbial inocula or commercial microbial agents because "if indigenous cultures do not accelerate the degradation rates, organisms enriched from different environments, grown in the laboratory, and not acclimated to a particular climatic or geographic location should be even less able to compete with the natural population."

Lee *et al*. (1997) conducted a 129-day field trial to compare the effect of four treatments on biodegradation of weathered Venture Condensate on a sandy beach in Nova Scotia, Canada. The four treatments (control, inorganic nutrient addition, a commercial bioremediation product, and addition of inorganic nutrients along with bioremediation product) as well as an unoiled control were replicated in a complete block design using 20 enclosures or plots. C_2-chrysene was used as the normalizing biomarker due to the low concentration of hopane in the condensate. PRP (PetrolRem, Inc.) was selected to be the representative commercial bioremediation agent in this study. This product is no longer listed in the current NCP Product Schedule. According to Lee *et al*. (1997), PRP contains mineral nutrients and nonpathogenic bacteria within spherical particles made from plant derived natural products (beeswax) and exhibits both bioaugmentation and biostimulation properties. The agricultural fertilizer used in this study was a mixture of granular forms of ammonium nitrate (N:P:K: 33-0-0) and triple super phosphate (N:P:K: 0-46-0). The study showed that an average of 11.0% of the n-alkanes remained in the oiled control plots, and only 0.1% of the oil remained in the enclosures treated with inorganic nutrients alone; 5.4% of the alkanes were found in the plots treated with inorganic nutrients and PRP, and 25.3% remained in the plots treated with PRP alone. The results indicate that periodic addition of inorganic nutrients was the most effective strategy for enhancing oil degradation and that the full potential of the bioremediation product was limited by nutrient availability. This field trial demonstrated that adding the bioremediation product did not perform better in terms of enhancing alkane degradation than applying inorganic agricultural fertilizers alone.

Several other possible reasons for the failure of inocula in degrading contaminants in nature were summarized by Goldstein *et al*. (1985), which include: (1) the concentration of the contaminant may be too low to support the growth of the inoculated species, (2) the natural environment may contain substances inhibiting growth or activity of the inocula, (3) the growth rate of the inoculated species may be limited by predation such as protozoa, (4) the added species may use other substrates in nature rather than the targeted contaminants, and (5) the seeded microorganisms may be unable to move through the pores of the sediment to the contaminants.

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. The two products basically contain the same bacterial cultures and nutrients (Hozumi *et al*., 2000). The observed visual effects might have been due to physical or chemical processes such as surfactant action associated with the products (Swannell *et al*., 1996) or sinking.

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.

2.3 Application of Biostimulation Products

Biostimulation involves the addition of rate-limiting nutrients to accelerate the biodegradation process. 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. Theoretically, approximately 150 mg of nitrogen and 30 mg phosphorus are consumed in the conversion of 1 g of hydrocarbon to cell material (Rosenberg and Ron, 1996). Therefore, a commonly used strategy has been to add nutrients at concentrations that approach a stoichiometric ratio of C:N:P of 100:5:1. However, the practical use of these ratio-based strategies remains a challenge. Particularly, in marine shorelines, maintaining a certain nutrient ratio is impossible because of the dynamic washout of nutrients resulting from the action of tides and waves. A more practical approach is to maintain the concentrations of the limiting nutrient or nutrients within the pore water at an optimal range (Bragg *et al*., 1994;

Venosa *et al*., 1996). It is overwhelmingly evident from the literature that common agricultural fertilizers would be the first choice of nutrient additives since they are both inexpensive and readily available. However, because water-soluble nutrients are amenable to rapid washout, attempts have been made to design nutrient delivery systems that overcome the washout problems and to enhance nutrient availability for oil biodegradation. As a result, a few commercial biostimulation products have been developed. Field studies on both common agriculture fertilizers and commercial biostimulation agents are reviewed in the following section.

2.3.1 Common agricultural fertilizers

2.3.1.1 Water-soluble fertilizers

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). One of the early field trials using common commercial fertilizers was carried out in Spitsbergen, Norway in 1976 (Sendstad, 1980). Forcados unweathered crude oil was released at a rate of 10 L/m^2 on each of two 10 m^2 plots. One plot served as an oiled control, and the other was treated with an unspecified commercial fertilizer at a concentration of 0.1 kg/m^2. A marked increase in microbial respiration rate was observed in the fertilized plot compared to the control plot, suggesting that the application of fertilizer stimulated oil degradation. However, the conclusion was questionable due to the inadequate control and the lack of replicate plots in this study (Venosa, 1998).

Researchers from Fisheries and Oceans-Canada (Lee and Levy, 1987; Lee and Levy, 1989; Lee and Levy, 1991; Lee and Trembley, 1993; Lee *et al*., 1995; and Lee *et al*., 1997) conducted a series of field tests to investigate the effect of different types of fertilizer and different delivery strategies in a low energy, sandy beach or in a salt marsh. Their studies demonstrated that biostimulation using periodic addition of inorganic fertilizers (ammonium nitrate and triple super phosphate) increased the rate of oil removal from beaches as measured by changes in oil composition relative to conserved biomarkers such as C_2-chrysene and/or the decline in the n-C_{17}/pristane and n-C_{18}/phytane ratios (Lee and Levy, 1987 and 1989). Another study involved periodic addition of water-soluble fertilizer granules (ammonium nitrate and triple super phosphate) in an attempt to enhance biodegradation of waxy crude oil in a low-energy, sandy beach and in a salt marsh (Lee and Levy, 1991). Two concentrations of the NH_4NO_3 were tested (0.34 and 1.36 g/L sediment). The oil used was Terra Nova crude at two different levels (0.3 and 3.0%). Results from the sandy beach showed that at the lower level of oil contamination, no enhancement by fertilizer was achieved. However, at the higher oil contamination level, substantial oil degradation occurred in the fertilized plots compared to the unfertilized ones. Results in the salt marsh were the exact opposite. Enhancement by fertilizer was significant at the 0.3% contamination level, but no enhancement occurred at 3% oil contamination, which was attributed to the penetration of oil into the anaerobic zone where little degradation is expected. Another field study conducted by Lee *et al.* (1995) compared the performance of inorganic nutrients with organic fish bone-meal fertilizer. These results showed that the organic fertilizer had the greatest effect on microbial growth and activity due to the presence of a readily biodegradable form of carbon in the bone meal, while the inorganic nutrients were much more effective in stimulating crude oil biodegradation.

Recent studies found that the oil biodegradation rate depends on the nutrient concentrations in the pore water of the sediments, which provides important guidance for nutrient applications (Bragg *et al*., 1994; Venosa *et al*., 1996). This finding may also explain why some earlier trials failed to demonstrate the effectiveness of nutrient application since the nutrient concentrations in the interstitial pore water had not been monitored and controlled in most of these studies. As mentioned earlier, the Delaware field study compared the effectiveness of biostimulation with inorganic mineral nutrients with that of microbial inoculation in enhancing the removal of crude oil. Venosa *et al.* (1996) found that maintenance of a threshold nitrogen concentration of 1-2 mg N/L in the interstitial pore water would result in close to maximum hydrocarbon biodegradation in a sandy beach. Another important conclusion from this study was that background nutrient concentrations at the contaminated site should be a determining factor in the decision to apply bioremediation. The background nitrogen concentration at the Delaware beach was high enough to permit close to maximum hydrocarbon biodegradation without the need to apply additional fertilizer despite the enhancement observed from nutrient addition. The enhanced effect, although statistically significant, was not substantial enough to have warranted a decision to implement bioremediation on a full-scale basis had there been a real spill at this site. This demonstrates that nutrient amendment might not always be necessary if sufficient nutrients are naturally present at a spill site in high enough concentrations to perform natural cleanup.

This conclusion is mitigated somewhat by the need to consider the resources at risk. For example, every spring in the Delaware Bay, horseshoe crabs come ashore to mate and lay eggs in the intertidal zone of the beaches. Migratory birds making their way from South America to Arctic Canada stop at this location to feed on the horseshoe crab eggs deposited in the sand. If an oil spill were to take place a few weeks before this feeding season occurs, biostimulation might be warranted despite the already high natural concentrations of nutrients present in the bay. Any amount of acceleration of the disappearance of oil in order to protect these sensitive bird populations would be justified. If a similar spill occurred in July, however, then bioremediation would likely not be justified since the natural attenuation rate is expected to be high enough to allow sufficient rates of biodegradation to take place with little likelihood of exposure of sensitive species to the oil spill.

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 experimental design was similar to the one used on the marine shoreline in Delaware Bay (Venosa *et al.*, 1996). The four oiled treatments included: (A) a natural attenuation control plot with no amendments; (B) a plot receiving ammonium nitrate and orthophosphate nutrients but with the wetland plants continually cut back to ground surface to suppress photosynthetic activity and growth; (C) a plot receiving the same nutrients as Treatment B but with the plants left intact, and (D) a plot similar to Treatment C but with only nitrate (no ammonium) serving as the nitrogen source. The results demonstrated that with respect to biodegradation of total alkanes and PAHs during the first 21 weeks of the investigation as measured by GC/MS analysis, only about 35% biodegradation occurred in all treatments on average, and no significant differences among any of the treatments were observed ($p > 0.05$). The study also found that better biodegradation occurred in surface samples in plots where the plants had been removed than in any of the core samples because of the oxic nature of the surface and the lack of competition for nutrients by the plant species. 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. A similar result was obtained from a field trial conducted in a Louisiana salt marsh (Shin *et al*., 1999), in which natural attenuation of crude oil was as effective as nitrogen (NH_4NO_3) amendment, and oxygen availability appeared to control the oil biodegradation process in salt marshes.

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. To achieve this under field conditions, especially in many estuarine and marine environments, frequent nutrient applications are required when using water-soluble fertilizers, therefore, resulting in more labor-intensive, costly, and physically intrusive operations.

2.3.1.2 Slow-release fertilizers

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. This approach may also cost less than adding water-soluble nutrients due to the need for less frequent applications. 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, particularly in combination with an oleophilic fertilizer, Inipol EAP22 (see next section) (Atlas, 1995; Pritchard *et al*., 1992; Swannell *et al*., 1996).

Several field studies have been carried out to evaluate the effectiveness of slow-release fertilizers on enhancing oil biodegradation. A field test was carried out to evaluate the performance of bioremediation by nutrient amendments for treating a mixture of Forties Crude Oil and Heavy Crude Oil stranded on Bullwell Bay, Milford Haven, UK, after the grounding of the Sea Empress in 1996 (Swannell *et al*., 1999a&b). A randomized block design with triplicate treatments was used to test the efficacy of two biostimulation amendments: a weekly application of an inorganic nutrient solution ($NaNO_3$ and KH_2PO_4) and a single application of a slow-release fertilizer. The slow release fertilizer pellets consisted of a mixture of inorganic nutrients (15-4.8-13) with a coating derived from soya oil. Oil components were measured using GC/MS, and hopane was used as a biomarker. Results showed that the addition of both liquid inorganic nutrients and the slow release fertilizer significantly stimulated natural oil biodegradation. After two months, the oil in the nutrient-amended plots was 37% more degraded than that found in the control plots. Because the slow-release fertilizer was much less labor-intensive to apply, the study concluded that the application of slow-release fertilizers might be a cost-effective method for treating low-energy, oil-contaminated shorelines.

A field study conducted in a tropical marine wetland in Australia also showed promise for the application of slow-release fertilizers (Burns *et al*., 2000). In this study, four oiled treatments (two types of oils with and without the bioremediation treatments) and two unoiled controls (enclosure and ambient controls) were tested. Four replicate plots were used for each treatment in the salt marsh plots. The bioremediation treatment consisted of sprinkling Osmocote Tropical fertilizer at 0.15 kg m^{-2} 40 hours after oiling. Other than total petroleum hydrocarbons (TPHs), individual alkanes were also analyzed using GC-FID, and phytane was used as a biomarker. The results indicated that although the predominant oil removal processes were evaporation and dissolution, the addition of the fertilizer to the salt marsh plots stimulated the degradation of the medium range crude oil (Gippsland), resulting in about 20% more oil loss as compared with the untreated plots after 9 months. However, the nutrient amendment did not significantly affect the rate of loss for the heavier Bunker C oil, confirming that the efficacy of bioremediation is somewhat dependent on the type of oil to be treated.

Another field trial involving the application of a slow-release fertilizer was carried out on an Arctic beach (Prince et al., 1999). Four treatments (tilled, tilled & fertilized, fertilized, and oiled control) were evaluated on four unreplicated plots along a gravel shoreline contaminated with a fuel oil (IF-30) near Sveagruva, Norway. A series of applications of various nutrient products were performed for both fertilized plots during the first two months of study. These nutrient products included a mixture of water-soluble fertilizers, yeast extract, and a slow-release formulation (Inipol SP1, CECA, Paris La Defense), which contained 18% NH_4 –N and 1% P as P_2O_5. By 399 days after the first application of the fertilizers, changes in the chemical oil compositions (ratio of phenanthrene to the dimethyl- and ethyl-phenanthrenes) suggested to the investigators that biodegradation was significant. However, the extent of this preferential removal of phenanthrene was not different among the two fertilized plots and the oiled control (no statistical analysis was possible because of the lack of replicate plots). The authors concluded that the biostimulation application was effective in enhancing oil biodegradation based only on increased microbial activity (oxygen consumption) and biomass growth. This conclusion, which is based on indirect evidence, may be somewhat questionable due to lack of replication in the experimental design and the attempt to determine too many factors in a limited number of tests, resulting in the confounding of different effects (e.g., the effect of yeast extract on enhancing oxygen consumption).

The efficacy of biostimulation also depends on environmental factors such as temperature, shoreline energy, substrate, and background nutrient concentrations. A field study conducted by Lee *et al*. (1993) indicated that the effectiveness of specific nutrient formulations might be influenced by temperature conditions. The study investigated the efficacy of water-soluble inorganic fertilizers (ammonium nitrate and triple super phosphate) and a slow release fertilizer (sulfur-coated urea) to enhance the biodegradation of a waxy crude oil in a low energy shoreline environment. The results showed that at temperate conditions above 15°C, the slow-release fertilizer appeared to be more effective in retaining elevated nutrient concentrations within the sediments and enhancing oil degradation than water-soluble fertilizers. However, lower temperatures were found to reduce the permeability of the coating on the slow-release fertilizer, and as a result nutrient release rates were suppressed. Water-soluble fertilizers, such as ammonium nitrate, were recommended under these temperature conditions.

Oudot et al. (1998) evaluated the influence of a slow-release fertilizer (Max-Bac, Grace-Sierra International) on the biodegradation rate of an Arabian Light crude oil contaminating an estuarine environment in the bay of Brest, France. A randomized block design with five replicate plots was used to examine the effects of two treatments (oiled control and fertilizer addition). The slow-release fertilizer (10% NO_3-N, 12% NH_4-N and 13% P_2O_5) was applied monthly over the 9 months field test. On average, 40% of the total oil, 83% of the aliphatics, and 55% of the aromatics were biodegraded in all the plots at the end of the experiment. No significant difference in oil biodegradation rates was observed between fertilized and non-fertilized plots based on GC/MS analysis using norhopane as the normalizing biomarker. These results were attributed to the high background levels of N and P at the study site. It was proposed that bioremediation by nutrient enrichment would be of limited use if background interstitial pore water levels of N exceed 1.4 mg/L, which is close to the levels found to be near optimum by Venosa *et al.* (1996).

The physical forms of fertilizers are also important in selecting appropriate nutrient products. Field trials conducted following the *Exxon Valdez* spill evaluated two types of slow release nutrients: isobutylidene diurea (IBDU) briquettes and Customblen granules. The application of the briquettes was problematic in regards to buoyancy of the briquettes and redistribution by tide and wave action (Glaser, 1994; Glaser *et al.,* 1991). The method used during the *Exxon Valdez* spill involved packing the briquettes in mesh bags tethered to steel bars driven into the beach subsurface. The poor distribution problem occurred by channeling of nutrients vertically down the beach rather than lateral spreading. In contrast, Customblen granules were evenly applied using a commercial broadcasting fertilizer spreader. Within two weeks after the fertilizer application, the area of cobble beach treated with Customblen appeared to be visibly cleaner than the untreated area (Pritchard *et al*., 1992).

The major challenge for the application of slow-release fertilizers is how best to control the release rates so that optimal nutrient concentrations can be maintained in the pore water over long time periods. For example, if the nutrients are released too quickly, they will be subject to rapid washout and will not be a lasting source. On the other hand, if they are released too slowly, the concentration will never build up to a level that is sufficient to support rapid biodegradation rates, and the resulting stimulation will be less effective than it could be. The field trials on of the shorelines of Prince William Sound showed that on certain beaches, Customblen granules were apparently washed away before any significant enhancement of bioremediation was recorded (*Swannell et al*., 1996). A recent mesocosm study by Sveum and Ramstad (1995) showed that a slow release nutrient (Max Bac) failed to demonstrate enhancement of oil degradation because the nutrient release rate was too low to affect oil biodegradation. Clearly, proper application of slow release fertilizers could be a promising bioremediation strategy for stimulating oil biodegradation.

2.3.2 Commercial biostimulation agents

Compared to microbial products, very few nutrient additives have been developed and marketed specifically as commercial bioremediation agents for oil spill cleanup. It is probably because common fertilizers are inexpensive, readily available, and have been shown effective if used properly. However, 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. Four of the 15-bioremediation agents listed on the NCP Product Schedule fall into this category (Table 2.1). The rationale for using oleophilic organic nutrients is that oil biodegradation mainly occurs at the oil-water interface, and since oleophilic fertilizers are able to adhere to oil and provide nutrients at the oil-water interface, enhanced biodegradation should result without the need to increase nutrient concentrations in the bulk pore water. This approach can also be used to overcome the problem of water-soluble nutrients being rapidly washed out. Field evaluation results for some of these products have been available in the peer-reviewed literature.

2.3.2.1 Inipol EAP22

Inipol EAP22 (Societe, CECA S.A., France) is currently listed on the NCP Product Schedule as a nutrient additive and probably the most well-known bioremediation agent for oil spill cleanup due to its use in Prince William Sound, Alaska. This nutrient product is a microemulsion containing urea as a nitrogen source, sodium laureth phosphate as a phosphorus source, 2-butoxy-1-ethanol as a surfactant, and oleic acid to give the material its hydrophobicity. The claimed advantages of Inipol EAP22 include: 1) preventing the formation of water-in-oil emulsions by reducing the oil viscosity and interfacial tension; 2) providing controlled release of nitrogen and phosphorus for oil biodegradation; 3) exhibiting no toxicity to flora and fauna and good biodegradability (Ladousse and Tramier, 1991).

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). Visual observation seemed to suggest that the bioremediation agent worked (Pritchard and Costa, 1991). However, the “window pane effect” observed within 2 weeks after application of Inipol to the beach was simply the result of oil having been lifted from the cobble and re-deposited in the interstitial sand matrix between and under the cobbles.

Using hopane as the biomarker, Bragg *et al*. (1994) showed that fertilizer application accelerated the rate of oil removal by a factor of approximately five-fold compared to natural attenuation. However, conclusions on the effectiveness of bioremediation in the Exxon Valdez experience are somewhat questionable, in part because the flawed experimental design was not based on sound statistical principles (Venosa, 1998). Major flaws included the lack of replication, inadequate sampling procedures, unequal treatment of controls and treated plots, and an attempt to determine too many factors in a limited number of tests, resulting in the confounding of different effects. The lessons learned from the Exxon Valdez project led to the replacement of "post *Exxon Valdez* excitement" with more scientifically-valid approaches (Mearns, 1997).

Extensive studies have been carried out under various field conditions on the efficacy of Inipol EAP 22 and have produced mixed results (Lee and Levy, 1989, Ladousse and Tramier, 1991; Sveum and Ladousse,1989). Lee and Levy (1989) conducted a field trial to investigate the effect of different types of fertilizers on enhancing the biodegradation of Scotian Shelf condensate and Hibernia crude oil. The study occurred in the intertidal zone of a low-energy sandy beach in Nova Scotia, Canada. The two nutrient products tested were Inipol EAP22 and a mixture of 10:1:0 agricultural fertilizer. The study demonstrated that biostimulation using periodic addition of the inorganic fertilizer increased the rate of oil removal from beaches as measured by changes in oil composition relative to conserved biomarkers such as C_2-chrysenes and/or the decline in the n-C_{17}/pristane and n-C_{18}/phytane ratios. In contrast, the addition of the oleophilic fertilizer, Inipol EAP 22, did not enhance oil degradation.

The effectiveness of Inipol EAP22 also depends on the characteristics of the contaminated environment, such as action of wave and tide, and the effect of different sediment types. 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. Inipol EAP 22 was found to be more effective in coarse sediments than in fine sediments due to the difficulty in penetration for the oleophilic fertilizer in fine sediments (Sveum and Ladousse, 1989), although stronger evidence is needed to confirm this suggestion. Variable results have also been produced regarding the persistence of oleophilic fertilizers. Some studies showed that Inipol EAP 22 could persist in a sandy beach for a long time under simulated tide and wave actions (Santas and Santas, 2000; Swannell *et al*. 1995). Others found that Inipol EAP22 was rapidly washed out before becoming available to hydrocarbon-degrading bacteria (Lee and Levy, 1987; Safferman, 1991).

2.3.2.2 BIOREN

Researchers from European EUREKA BIOREN program recently conducted a field trial in an estuary environment to evaluate the effectiveness of two bioremediation products (BIOREN 1 and 2) (Le Floch *et al*., 1997 and 1999). The EUREKA BIOREN project was an international effort to develop commercial nutrient products able to enhance hydrocarbon biodegradation on contaminated shorelines. The two nutrient products are derived from fish meals in a granular form with urea and super phosphate as nitrogen and phosphorus sources and proteinaceous material as the carbon source. The major difference between the two formulations was that BIOREN 1 also contained a biosurfactant. To reduce the effect of physical removal of oil and nutrients, the study was conducted on a sheltered sandy beach in a small estuary in Brittany, France. A light Arabian crude oil was used as the model contaminant in this field trial. Four treatments (unoiled control, oiled control, BIOREN 1, and BIOREN 2) were randomly assigned to four experimental plots (5m x 5m), and four smaller oiled control plots (2m x 3m) were also set up along side the treated plots. The nutrient products were applied twice over the 4-month study (once immediately after oiling and again two weeks after). The results showed a "starter effect" for the BIOREN 1 formulation: biodegradation was significantly enhanced during the first five weeks of the experiments based on the analysis of the nC_{17}/pristane and nC_{18}/phytane ratio. However, after five weeks, the enhancement was reduced and "significant differences" were no longer observed between treatments at the end of the test. The authors concluded that the BIOREN 1 hold promise for accelerating microbial activity immediately after an accidental oil spill.

The results seem to suggest that the biosurfactant in BIOREN 1 was the most active ingredient that contributed to the increase in oil degradation rates since BIOREN 2, which contained no surfactant, was not effective in that respect. The biosurfactant could have contributed to greater bioavailability of hydrocarbons to microbial attack. It would have been better if the investigators had used replicate treatments because this would have enabled them to calculate experimental error. Results would have been stronger in support of conclusions made.

Other studies on the effect of similar organic fertilizers derived from natural products also yielded mixed results. A field trial conducted by Lee *et al.* (1995a) compared the performance of inorganic nutrients with an organic fish bone-meal fertilizer on the biodegradation rates of Venture Condensate in a sandy beach environment. The results showed that the organic fertilizer had the greatest effect on microbial growth and activity, while the inorganic nutrients were much more effective in stimulating crude oil biodegradation. One of the problems with these types of fertilizers is that they contain organic carbon, which may be biodegraded by microorganisms in preference to petroleum hydrocarbons (Lee *et al*., 1995a; Swannell *et al*., 1996), which may lead to undesirable anoxic conditions (Lee *et al*., 1995b; Sveum and Ramstad, 1995).

2.3.2.3 Oil Spill Eater II® (OSE II)

Oil Spill Eater II® (Oil Spill Eater International, Corp.) is another nutrient product listed on the NCP Schedule (U.S. EPA, 2002). This product is listed as a nutrient /enzyme additive and consists of "nitrogen, phosphorus, readily available carbon, and vitamins for quick colonization of naturally occurring bacteria". A field demonstration was recently carried out at a bioventing site in a Marine Corps Air Ground Combat Center (MCAGCC) in California to investigate the efficacy of OSEII for enhancing hydrocarbon biodegradation in a fuel-contaminated vadose zone (Zwick *et al*., 1997). The selection of OSEII was base on the findings from a previous microcosm study, in which various amendments were evaluated by monitoring microbial respiration using soils collected from the site. The results suggested that fertilizer amendment, not bioaugmentation, might be cost-effective for accelerating biodegradation rates at this field site (Alleman and Foote, 1997). Although groundwater environments and subsurface hydrocarbon contamination are generally not within the scope of this review, this article was included because it is the only peer-reviewed paper available on a NCP-Schedule-listed bioremediation nutrient additive, other than numerous publications on Inipol EAP 22.

At beginning of the test, air was pumped into vent wells for 36 hrs to achieve oxygen concentrations of over 20% at the site. Groundwater was injected at 20 ft below ground surface (bgs), while OSE II solution was pumped to 30-40 ft. bgs through two monitoring points. OSE II was also injected into two monitoring points in an uncontaminated area of the site to act as product controls. An On-line Environmental Monitoring System (OEMS) was used to conduct in-situ measurement of O_2 and CO_2 at depths of 10, 20, 30, and 40 ft bgs. Hydrocarbon degradation rates (as mg hexane per kg of soil per day) were calculated based on oxygen utilization rate, and CO_2 data were used to verify biodegradation. The effect of OSE II was monitored for about three months after the initial application and the results were compared to those obtained before the OSE II injection when irrigation was conducted for over a year.

The oxygen and CO_2 data showed that an increase in respiration rates occurred shortly after the addition of OSE II, especially at the 30-ft bgs level, indicating an increase in microbial activity as a result of OSE II addition. The extent of the rate increase was much higher than that measured at the uncontaminated background site receiving OSE II, suggesting the BOD associated with the product may not be the main cause for the increase in the respiration rates at the contaminated site. However, the respiration rates at both 10 ft bgs (oiled-control) and 20 ft bgs (groundwater irrigation) levels at the contaminated site were also higher than the pre-test levels, suggesting that factors other than the OSE II addition might also be responsible for the increase in oxygen utilization rates. These factors could be the pre-aeration at the beginning of the study (the control site had not been pre-aerated as the test site was), changes in environmental conditions, or differences in geology at the various depths., No statistical analysis was carried out to test the significance of the findings in this study. Although respiration rates may be an indirect measure of product effectiveness, proof of effectiveness comes from measurement in decline of hydrocarbons, which was not discussed in the report. Although this field trial suggested that irrigation and OSE II addition might have enhanced microbial activities in the deeper soils at the site, the report was inconclusive in regards to direct evidence that hydrocarbons were degraded.

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. Successful application of bioremediation products will always require appropriate experimental design, testing, and evaluation based on the specific conditions of each contaminated site.

2.4 Summary

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. Important findings and lessons learned from these studies are summarized as follows.

- Bioaugmentation appears to have little benefit for the treatment of spilled oils in an open environment. Microbial addition has not been shown to work better than nutrient addition alone in many field tests. However, application of bioaugmentation products could still have some potential in the treatment of specific oil components or isolated spills in confined areas, although more evidence is still required to verify this notion.

- Bioremediation with addition of nutrient products has been proven to be an effective tool to treat certain aerobic oil-contaminated marine shorelines. Typically, it is used as a polishing step after conventional mechanical cleanup options have been applied, although it could also be used as a primary response strategy if the spilled oil does not exist as free product and if the contaminated area is remote enough not to require immediate cleanup or not accessible by mechanical equipment.

- Effectiveness of biostimulation is also highly site-specific. When oxygen is not a limiting factor, one of the key factors for success is to maintain an optimal nutrient level in the interstitial pore water. In other words, background nutrient concentrations at the contaminated site should be a determining factor in the decision to apply bioremediation, and biostimulation might not always be necessary if sufficient nutrients are naturally present at a spill site in high enough concentrations to permit effective microbial treatment. Availability of oxygen is often the limiting factor in wetland environments, where addition of nutrient products has not been successful in enhancing oil biodegradation. If the oil is in the aerobic zone of a wetland sediment (upper few mm) and if background nutrients are low, then biostimulation may still be an effective cleanup strategy. Even if oil has penetrated into the anaerobic zone, biostimulation may at least allow for faster recovery of the wetland vegetation.

- Nutrient products have shown variable effectiveness, depending on oil properties, the nature of the nutrient products, and the characteristics of the contaminated environments. In general, commercial oleophilic nutrient products have not shown clear advantages over common agricultural fertilizers in stimulating oil degradation.

- As this review has pointed out, many field tests have not been properly designed, well controlled, or correctly analyzed, leading to skepticism and confusion among the user community when selecting response options (Venosa, 1998). Future field studies should devote more energy and investment to adopting scientifically valid approaches and acquiring the highest quality data possible.

3 Assessment of Oil Bioremediation Products: Non-Peer Reviewed Literature

As indicated in the previous chapter, 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 has also been conducted. A comprehensive review of this information is presented in this chapter, which includes the review of government agency reports, vendor reports, and vendor client reports. Section 3.1 presents a thorough review of field trials of bioremediation products based on various government agency reports. Section 3.2 summarizes the results of an information collection effort from manufacturers and vendors of bioremediation products and provides an assessment of the efficacy of some bioremediation agents based on the gathered information. The potential of using bioremediation products in the areas of non-point source such as storm water runoff countermeasures and bilge oil treatment will be discussed in Section 3.3.

3.1 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. For example, EPA is in charge of coordinating oil spill response in inland environments; the USCG coordinates oil spill prevention and response in the coastal zone; and National Oceanic and Atmospheric Administration (NOAA) as well as EPA provide national coordination with respect to scientific support. In this project, many of the governmental agencies that have likely been involved in evaluating and using bioremediation approaches in oil spill responses were contacted and/or their publication websites were searched. These governmental agencies include U.S. EPA Oil Spill Office, USCG, NOAA, Department of Energy, Department of Interior, the U.S. Navy, U.S. Army Corps of Engineers, and various state agencies. From this search, a number of government reports pertaining to the use of commercial bioremediation products were obtained. Although some of this literature has already been covered in other reviews, such as Swannell *et al.* (1996), Venosa (1998), and Zhu *et al*. (2001), they will still be discussed within the context of effectiveness of commercial bioremediation agents. Again, all this literature will be reviewed based on the two main bioremediation approaches, bioaugmentation and biostimulation.

3.1.1 Application of bioaugmentation products

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. The test was conducted 8 days after the spill, which resulted in the release of approximately 700,000 gallons of catalytic feedstock (a partially refined oil). Four plots were used in selected areas of Marrow Marsh: two treated with a commercial bioaugmentation product (Alpha BioSea^{TM}, Alpha Environmental, Inc.), and two left untreated as controls. The product consisted of a bacterial culture in a cornstarch carrier and a nutrient/micronutrient mixture. It was prepared by premixing with ambient brackish water, and a diluted stock solution was sprayed on the two marsh test plots. 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. During only a 4-day monitoring period, results of the chemical analysis indicated no apparent difference in the extent of oil biodegradation between treated and untreated plots, although no statistical analysis was performed. Mearns speculated that either the oil had too low a content of degradable components or insufficient bioremediation agent was used. However, the major deficiency of this study was that 4 days were insufficient for reaching any convincing conclusion on the effectiveness of bioremediation. Biodegradation is a relatively slow process, and usually weeks or months are needed before significant microbial activity may take effect. 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.

In another NOAA report, Hoff (1991) described a bioaugmentation project along a California shoreline. On October 31, 1990, a well blowout offshore of Seal Beach, CA, released approximately 400 gallons of crude oil resulting in the contamination of 2-3 acres of marsh grasses in the Seal Beach National Wildlife Refuge. A bioremediation treatment that consisted of the hand spraying of a microbial product used in wastewater treatment plants (INOC 8162) and a commercial fertilizer (Miracle Gro 30-6-6) was carried out one week after the incident, followed by an application of the fertilizer alone two weeks later. Oil degradation was monitored based on the measurements of ^{14}C-mineralization and most probable number counts of bacteria. No difference was observed between treated and untreated salt marsh plots 35 days after the initial treatment, suggesting neither bioaugmentation nor biostimulation with nutrient addition worked in this case. However, no reporting of nutrient concentrations was provided, so it was difficult to determine if sufficient nutrients were available to allow biodegradation to take place. Nonetheless, the result is consistent with the finding by others that oxygen availability is likely the limiting factor for oil biodegradation in wetland environments (Shin *et al*., 1999; Simon *et al*., 1999; Venosa *et al*., 2002).

A pilot-scale project was carried out recently to test the efficacy of a bioremediation procedure in treating soils contaminated by petroleum oils and lubricants (POLs) at an Army installation at Fort Hood, TX (U.S. Army, 1999). POLs are common contaminants on Department of Defense (DoD) installations, and the U.S Army is increasingly being asked to comply with more stringent regulations for disposal of these wastes. The treatment procedure tested in this study included the addition of a commercial bioremediation agent, BET BioPetro (BioEnviroTech, Inc.), at 1 lb/yd^3 of contaminated soil and an agricultural fertilizer (24-8-8) at a rate of 0.5 lb/yd^3. After the addition of both microbial and nutrient products, the contaminated soil was tilled and then watered at a rate of 1.5 in/week. BET BioPetro is one of the bioremediation agents listed on the NCP Product Schedule (Table 1.1). According to the vendor's description, BET BioPetro is "a powder containing granules of bacterial product formulated to provide performance in the bioremediation of heavy refined and crude hydrocarbon contaminants in both soil and water environments" (U.S. EPA, 2002). In this field trial, the bioremediation procedure described above was tested for six months at three sites contaminated by JP8 fuel oil, and the results were successful in terms of oil degradation based on

TPH analysis. For example, six months after the initial treatment was applied, TPH concentrations decreased dramatically from the initial values of 10,100 – 13,100 mg/kg to the final results of 195 – 1,170 mg/kg at Site 2. Treated soils were able to meet the reuse requirements for the final disposal to sanitary landfills. Unfortunately, no control was set up during this study; therefore, no conclusion could be reached in regard to whether this apparent drop in oil concentration was due to the addition of BET BioPetro or any other treatments (i.e., nutrient addition, tilling, or watering). The lesson from this project again demonstrates how critical a proper experimental design is in testing the effectiveness of bioremediation.

Bioremediation experience at U.S. Navy's Naval Facilities Engineering Service Center (NFESC) also suggested that bioaugmentation is generally not needed for treating fuel/oil contaminated soils because of the ubiquity of hydrocarbon degraders in nature. However, according to personnel at the NFESC, microbial amendment may have limited use for treating specific contaminants or in specific environments. An example was given of the potential usefulness of a specific microbial product. A bioremediation project that involved adding a commercial bioaugmentation agent to a biopile was conducted at a naval air station (NAS) in Fallon, Nevada in 2002 (personal communication with personnel at the NFESC). The soils were contaminated with a mixture of fuels (mostly aged gasoline and JP-5). Sulfate and sulfide levels in these soils were also very high. When aeration was imposed by means of a vacuum pump, the soil temperature increased dramatically to about 70°C, probably due to high oxidation rates of the sulfides. The naval investigators surmised that because of the high temperatures caused by the forced aeration conditions, bioaugmentation might be helpful to enhance hydrocarbon biodegradation in a subsequent treatment step. A bioaugmentation treatment was carried out by Pintail Systems (Denver, CO), which involved the application of a product consisting of a mixture of microbial isolates from soils at the site plus some cultures isolated from an acid mine drainage site. Three months after the application of the microbial product, TPH concentrations in the soils dropped dramatically from pre-treatment levels of about 2,000 mg/kg to about 200 mg/kg. Again, no control pile was established to help demonstrate the effectiveness of the bioaugmentation treatment. Therefore, this result in no way proves or even suggests that the bioaugmentation product used was effective. It is common that compost and biopiles can be highly effective in treating organic waste products, and temperatures that are attained can reach very high levels.

3.1.2 Application of biostimulation products

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. Hoff (1991) summarized a field trial conducted by Exxon Research & Engineering Company with technical support from DuPont Environmental Remediation Services, on Prall's Island, New Jersey. The study was carried out between September and December 1990 to assess the effectiveness of a slow-release fertilizer (Customblen, Sierra Chemicals) following a pipeline leak of No.2 fuel oil on a beach at the Prall's Island bird sanctuary in January 1990. The slow-release fertilizer was placed in two shallow trenches about 4 to 6 inches bellow the surface in the intertidal zone. A no-treatment control plot was set up next to the treated zone. According to Hoff (1991), the results were inconclusive, in part due to the high variability in the TPH levels within the treated and control areas and the lack of replicated plots. Hoff also suggested that either cross contamination of nutrients might have occurred between the two treatment zones or high background nutrient levels might have masked effects of nutrient addition. The ammonium concentration in the interstitial pore water at the control plot was about 1 mg/L, which is close to the minimum nitrogen level in pore water that would permit close to maximum hydrocarbon biodegradation (Venosa *et al*., 1996). It should also be noted that in a company report, prepared by Exxon Research & Engineering Company and DuPont Environmental Remediation Services (Madden *et al*., 1991), a different conclusion was reached based on the same experimental data. The company report indicated that "fertilizer addition clearly accelerated the rate of biodegradation", although it admitted that the controls were not sufficient to provide a clear side-by-side comparison between treated and untreated zones. The conclusion was based mainly on the marked decrease in average TPH within the treated plots three weeks after the nutrient amendment. Unfortunately, neither of these two reports provided any statistical analysis in regard to the experimental data to support their conclusions. Of course, statistical analysis would have been impossible anyway since neither the control plots nor the treated plots were replicated and randomly situated in the intertidal zone. Again, this controversy demonstrates the importance of proper experimental design and data interpretation in bioremediation evaluation. 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. The results showed that nutrient amendments have generally been successful in reducing hydrocarbon levels in the tundra soil. For example, in August 1989, a crude oil spill from a leaking valve on a production line occurred in the North Slope oilfields. After pooled oil was vacuumed, fertilizers (8-32-16 at 215 lbs/acre and 34-0-0 at 185 lbs/acre) were applied during spring/summer of 1990 and 1991, respectively. Following the nutrient treatment, the mean soil TPH concentration decreased 92% by 1993 and 96% by 1996. Similar results were obtained in two other cases that used the same fertilizers. In another case, a field trial was carried out to evaluate different revegetation methods following a crude oil spill from a check valve station near Prudhoe Bay. Five treatments were established: 1) cover with clean material, 2) remove contaminated material and replace with clean material, 3) till with a concrete rake, and 4) apply an unidentified oil degrading bacterial product, 5) no treatment. These plots were then seeded with vegetation and fertilized with a 14-30-14 agricultural fertilizer. The study found that the most effective treatment in terms of both oil degradation and grass yield was the combination of tilling and fertilization. The result also suggested that biostimulation with nutrient addition alone was more effective than microbial seeding in this arctic tundra environment.

3.2 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. A summary of the results of this information collection and a comprehensive review of these non-peer reviewed articles are presented in this section.

3.2.1 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 (see Appendix A, Initial Letter Calling for Information). A follow-up letter listing the detailed information requested was then issued to each company that positively responded to our initial letter (see Appendix B, Follow-Up Letter to Participating Companies). A total of eight vendors of bioremediation products were willing to participate in this investigation and submitted at least some the information requested. The name, major bioremediation products, and contact information of these participating companies are listed in Table 3.1.

Table 3.1 List of Companies That Participated in This Review

Manufacture or Vendor	Bioremediation Product(s)	Contact Information
Enviro-Zyme, Inc.	BR, formerly ENVIROZYME BR	P.O. Box 169 Stormville, NY 12582 Tel: 1-800-882-9904 info@envirozyme.com
Forrester Environmental Technologies Corp. (FET Group)	BioCATalystIOS-500	P.O. Box 3652 Vero Beach, FL 32964 1-407-758-9033 envirotec@earthlink.net
Garner Environmental Services, Inc.	Petro-Clean	1717 West 13th Street Deer Park, TX 77536 1-800-424-1716 wsbiosolve@aol.com
Industrial Wastewater Solution	IOS-500	P.O.Box 157 Sebastopol, CA 95473 1-707-824-1282 IWS@sonic.net
Medina Agriculture Products Co., Inc.	Medina Microbial Activator Bio-D Nutrients Hydrocarbon D-Grader	P.O. Box 309 Hondo, TX 78861 1-830-426-3011 medina@medinaag.com
Petrol Rem, Inc.	PRP (Petroleum Remediation Product) (a/k/a WAPED)	2275 Swallow Hill Road Bldg 2500 Pittsburgh, PA 15220 1-800-246-2275 info@petrolrem.com
Verde Environmental, Inc.	Micro-Blaze	7309 Schneider Houston, TX 77093-8501 1-800-626-6598 bscogin@micro-blaze.com
WMI International, Inc.	WMI-2000	4901 Milwee Suite 109 Houston, TX 77092 1-800-460-45074 wmi@wt.net

3.2.2 Summary of case studies submitted by vendors

A complete list of materials submitted by the eight vendors is shown in Table 3.2. A brief description of their major bioremediation product(s) and a summary of selected case studies for each company are presented here, following the alphabetical order of the companies' names. 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.

3.2.2.1 Enviro-Zyme, Inc.

Product(s)

BR (formerly ENVIRO-ZYME BR) is the major oil bioremediation product manufactured by Enviro-Zyme, Inc. This product was listed on the NCP Product Schedule as a biological addictive when this report was written. According to the company, "BR" contains sufficient types of microorganisms enriched to degrade oil, aliphatic, and aromatic chemical pollution in soil and aqueous environments. BR also contains nutrient additives and a surfactant. It is a dry solid product with a shelf life of 1 year. BR should be mixed with water and applied through a low-pressure spray nozzle.

Case studies

Information on several pilot-scale and full-scale applications of BR was provided by the company for each case, which included the use of BR to treat wastewater containing oil and grease in two full-scale cases and to treat a BTEX-contaminated soil in one pilot study. A summary of these relevant case studies is described as follows.

- Bioremediation of BTEX: A pilot test was conducted in central Florida to evaluate the effectiveness of BR in treating a surface soil contaminated by a hydrocarbon waste from a dye manufacturer. The primary contaminants in this waste were benzene, toluene, ethylbenzene, and xylene (BTEX). The results showed that 7 months after the application of BR, the removal efficiencies for the benzene, ethylbenzene and toluene were essentially 100%, while the xylene reached 88% removal. No control was reported in this study.

- Bioaugmentation treatment of oil and grease: A wastewater from a bus installation in Washington D.C. contained high levels of grease and oil. The average concentration of grease and oil in the waste holding tank was 21,800 mg/L, which was an unacceptable discharge to the municipal sewage system. BR was then added daily to the grease and oil holding tank in conjunction with aeration. After six weeks of the treatment, the grease and oil level decreased to an average of 1,200 mg/L. Similarly, BR was also used to assist in treating a wastewater containing oil and grease from a New York railroad yard. The industrial wastewater was normally treated in an aeration lagoon. However, during the winter season, treatment efficiency declined, and the lagoon could no longer meet the State Pollution Discharge Elimination System (SPDES) Permit limits. The wastewater used to be bypassed to a holding basin until spring. However, with the addition of BR to the influent of the aeration lagoon at a rate of 2 pounds per day during the winter season, the effluent has successfully met the SPDES permits for BOD, TSS, and grease & oil, and has been allowed to discharge into the Hudson River all year-round.

3.2.2.2 Forrester Environmental Technologies Corp. (FET Group)

Product(s)

BioCATalyst (a.k.a. Sheen Solution, Bio Cat 2001, BIOCAT VFB) is a bioremediation product custom manufactured for FET Group by Biocat VFB Solutions Company, Inc., Toccoa, GA. This product is a kelp extract modified by a surface-active agent and other natural materials. The main active ingredient is a cytokinin extract, which is a plant hormone known to be a plant growth enhancer.

According to a FET Group's technical bulletin, BioCATalyst "works in two ways on oil spills": dispersion and biostimulation. The product contains a natural plant surfactant and emulsifier, which acts to break up oil into droplets, suspending the droplets into the water column. Indigenous bacteria are claimed to be biostimulated by the cytokinin such that their metabolic processes are greatly enhanced, resulting in much quicker biodegradation of substrates. BioCATalyst is normally applied as a fine spray. It is also recommended to be mixed on site with nutrient solutions supplied by the manufacturer.

Case studies

Chemical analysis reports on two full-scale applications of BioCATalyst were provided by FET Group, which involved treating petroleum hydrocarbon contaminated water in Florida. One of the reports came with a one-page case description, which is summarized as follows.

- At an orange grove in central Florida, a leak of fuel tanks used for orange blossom heaters led to heavy soil contamination by diesel and gasoline. After the removal of the contaminated soil, groundwater flowed into the excavation and also became contaminated with petroleum hydrocarbons. The 163,000 gallons of water was first treated with the chemical oxidant potassium permanganate. However, this approach was unable to meet the Florida DEP requirements. BioCATalyst was then applied at a rate of approximately 10 gallons per day over a period of 14 days. The analysis results (EPA method 610/8100) showed that hydrocarbon concentrations decreased dramatically after the treatment, and the site was able to meet the required State levels. For example, the concentrations for benzene, xylene, and naphthalene were reduced from 42, 335, and 22 μg/L to 1, 1 and 5 μg/L , respectively, in 14 days. Similar results were obtained using BioCATalyst for the treatment of another open pit of contaminated aquifer water in a second analytical report.

3.2.2.3 Garner Environmental Services, Inc.

Product(s)

Garner Environmental Services, Inc. is a primary distributor of "Petro-Clean", a product manufactured by Alabaster Environmental Corp., Pasadena, TX. According to the product catalog, Petro-Clean contains surfactant, nutrients, and hydrocarbon degrading bacteria. This product is currently listed on the NCP Product Schedule as a surface-washing agent based on one of its main active ingredients – a surfactant. On the other hand, it is also marketed as a bioremediation agent by vendors since it also contains active microbes (estimated by the manufacturer at approximately 50 billion per gallon). The main microbial species are naturally occurring *Bacillus* spores. Petro-Clean is normally applied through power washers or even garden type sprayers in diluted solution. More information in regard to this product can be found on the web site of EPA Oil Spill Program (http://www.epa.gov/oilspill).

Case studies

The manufacturer provided information on four full-scale applications of Petro-Clean. Three of these cases involved the cleanup of petroleum hydrocarbon contaminated soil, and the other concerned the treatment of creosote-contaminated soil. TPH or creosote concentrations before and after Petro-Clean treatments were provided for all the cases, which showed apparent reduction of the contamination levels after the treatments. One of the case studies that involved the cleanup of petroleum hydrocarbons and included a detailed sampling layout is summarized as follows.

- Bioremediation of a Petroleum Compressor Station: A severe petroleum hydrocarbon spill occurred due to a leak from three compressor units. The soil/gravel site was approximately six acres with heavy contamination on three acres. The bioremediation treatment involved the following techniques: 1) wet vacuum removal of surface liquid contamination; 2) surface cleaning with Petro-Clean; 3) subsurface injection with Petro-Clean; and 4) tilling in absorbents with Petro-Clean. Three sampling locations that covered various contamination levels were set up to monitor the performance of the treatment. Before the bioremediation operation, TPH concentrations for the three sampling sites were 11,000, 147, 000, and 130,000 mg/L, respectively. The results showed that within 2 to 6 weeks after the treatment, the levels at all three sites were reduced to a permitted TPH level, which was less than 10,000 mg/L.

3.2.2.4 Industrial Wastewater Solutions Corp.

Product(s)

Industrial Wastewater Solutions Corp. (ISW) is a corporate spinoff from International Organic Solutions (IOS), which is the manufacturer of a microbial product, called IOS-500 (US Patent #5.531.898). According to materials provided by ISW, which consults for IOS on bioremediation projects, IOS-500 is a blend of facultative bacterial species that use organic compounds as a primary food source and is effective in both aerobic and anaerobic environments. Based on the patent description (Wickham, 1995), IOS-500 also contains an enzyme mixture and an organic nutrient source. This product has been used for treatment of contaminated soils, remediation of contaminated bodies of water, and in agricultural, industrial and domestic wastewater treatment systems. The product is normally mixed with water for about 6 to 48 hours at ambient temperature to produce an acclimated mixture, and then applied to the contaminated environment.

Case studies

The manufacturer provided a table that lists 28 remediation cases using IOS-500 for treating hydrocarbon-contaminated soils. The information in the table includes clients' names and/or locations, types of oil/hydrocarbons, duration of the treatment, amount of the contamination, and the TPH levels before and after treatment. The package submitted by IWS also contained more detailed information on three cases that were conducted in Mexico for oil-spill cleanup using IOS-500, which included a one-page description for each case, pictures from the field, and letters of acceptance/acknowledgement from the customers. A brief summary of these three cases is presented as follows:

- Case #1: This case study was a demonstration at the request of a State-owned Oil Company in Mexico to prove the ability of the IOS product and process to provide rapid degradation of heavily contaminated areas. The project involved the remediation of 25 cubic yards of 50-year-old weathered crude oil in mud and soil. After 21 days of IOS-500 treatment, the TPH level was reduced by 89%, although no concentration data and sampling plan were described.

- Case #2: IOS-500 was used to cleanup 5,000 yd^3 of wetland soils contaminated by a 50-year-old spill of crude oil and tar. The contaminated soil was first excavated from the wetland and then mixed with IOS-500 using small front-end loaders, a water pump, and hoses. The TPH concentration for the untreated soil was 436,000 ppm on average (number of samples and replication unknown). After 42 days of treatment, the soil TPH level was reduced to 4500 ppm.

- Cases #3: This case study involved the remediation of 10,000 yd^3 clay, sand and mud type soil contminated by a fuel oil. Using similar land treatment procedure as in Case #2, TPH concentrations were reported to decrease from 80,000 to 452 ppm within 60 days. It was also noted that this job was completed during the rainy season.

3.2.2.5 Medina Agriculture Products Co., Inc.

Product(s)

Medina bioremediation products include Medina Microbial Activator or Soil Activator, several nutrient formulations and microbial blends. Medina Microbial Activator is a liquid formulation derived from a controlled fermentation process and contains compounds that stimulate microbial activities. Nutrient products include NP-1000 and MS100-plus-NP for water phase bioremediation, and Bio-D for soil remediation. Bio-D is a liquid product containing organic humic substances, as well as inorganic nitrogen, phosphorus and potassium. Microbial products include a granular formulation (Hydrocarbon D-Grader) and a liquid formulation (HCD). Medina also offers a "Spill Response and Bioremediation Kit", which includes Medina Microbial Activator, Bio-D nutrients, Bio-C organic solvent, Hydrocarbon D-Grader, Petrosorb, plastic bags and sheet, and manual.

A general procedure (material concentrations unspecified or not reported) for using the kit to treat oil-contaminated soil was provided, which includes: 1) spray Bio-C solvent on the affected area if the spill is old; 2) till the soil to the depth of the oil penetration; 3) mix Hydrocarbon-D-Grader with water and spray over affected area, 4) mix Medina Microbial Activator and Bio-D together with water, spray over affected area, tilling the treated area, 5) add water on a weekly basis to keep treated area moist.

Case studies

Information on several pilot and full-scale applications of Medina bioremediation products was provided, which include two journal articles (Shafer 1991&1992) and three "Bioremediation Reports" (a two-page company fact-sheet) on the performance of Medina Soil Activator, and an abstract of a company report on a soil bioremediation project using an organic nutrient product. A summary of these case studies is described as follows.

- Pilot study on effectiveness of Medina Soil Activator: In October and November 1989, a seven week pilot study was carried out by Woodward-Clyde Consultants (Denver, CO) on a site in Oakland, CA to evaluate the effectiveness of three bioremediation methods for treating an oil contaminated soil. The three treatments included the addition of 1) Medina Soil Activator, 2) an emulsifier plus a multiple-nutrient fertilizer, and 3) an emulsifier, a fertilizer, and proprietary hydrocarbon-degrading microorganisms. The contaminated material was placed in three wooden frame plots (6.5 cubic yards per pile) for testing the three treatments respectively. No untreated-control was set aside in this study nor were any replicate plots set up. After the applications of these bioremediation products, each plot was tilled three times for the first four weeks. Two composited samples were taken from each plot for TPH analysis at 2^{nd}, 4^{th}, and 7^{th} weeks after the treatment. The results showed that all three treatment methods were able to reduce TPH concentrations from 605 mg/kg on average to at or below 200 mg/kg level. The Medina Soil Activator plot achieved the highest hydrocarbon removal with an average TPH concentration of 145 mg/kg by week 7. Because Medina Soil Activator was also the least expensive product, it was proposed as the choice of the treatment for the cleanup of this site.

- Bioremediation of diesel contaminated soil and tundra: In the early 1980s, a diesel fuel pipeline ruptured at a U.S. Air Force station in arctic Alaska. During the summers of 1989 and 1990, two bioremediation approaches were applied by Woodward-Clyde Consultants using Medina Microbial/Soil Activator to remediate the contamination. The first approach was to clean up the contaminated soil using a land treatment unit (LTU). The diesel-contaminated soil was excavated and moved to a nearby LTU. The average initial TPH concentration was 11,500 mg/kg (number of sample replicates unknown). A surfactant solution was first applied to the soil to make the oil more available. Then a diluted Medina soil activator was applied once every two weeks. The soil was also turned once a week to aerate the LTU. At the end of the six-week period, TPH in the soil was reduced by 42 percent. The treatment was resumed in the summer of 1990. The land treatment process achieved 75% TPH removal during these two treatment periods. To reduce the disturbance to natural vegetation in the arctic tundra, an *in-situ* treatment approach was also tested during the same period, which involved surface-spraying a surfactant and Medina soil activator. Overall TPH levels were reduced from an average of 19,000 mg/kg to 8,300 mg/kg (57% removal) based on analytical results of four composite samples from randomly selected locations in the affected area.

- Soil bioremediation using an organic nutrient product: Approximately 100 cubic yards of diesel-contaminated soil was treated using a Medina organic nutrient product in a Coca-Cola distribution facility in California. The contaminated soil was treated with this unspecified Medina nutrient product five times between November 2, 1990 and April 23, 1991 in a land treatment unit. TPH concentrations were reduced from an average of 240 ppm to less than 10 ppm (EPA method 418.1).

3.2.2.6 Petrol Rem, Inc.

<u>Product(s)</u>

Petrol Rem is the manufacturer of PRP (a/k/a WAPED), a bioremediation product listed as an *Enzyme Additive* on the current NCP Product Schedule. According to materials submitted by Petrol Rem, PRP consists of tiny spheres of treated wax, which contain nutrients. As mentioned earlier in Chapter 2, PRP also contains nonpathogenic bacteria within wax particles although they are mostly not oil degraders (Lee *et al*, 1997; NETAC, 1993b). When PRP is sprayed as a loose powder, it can absorb twice its weight of oil and form a physical matrix that floats on water, thereby preventing the pollutants from sinking and limiting the transport of oils to more sensitive areas. The matrix then provides an environment that uses naturally occurring microorganisms in the water to degrade the pollutant as well as PRP itself. The mechanisms of enhancing oil biodegradation for this product, however, are still not well understood (NETAC, 1993b).

PRP is available in three forms: 1) powder form for treating open water spills, 2) BioBoom for oil spills that require containment, and 3) BioSok for treating spills in enclosed areas such as boat bilges.

<u>Case studies</u>

Information on several pilot and full-scale applications of PRP products was provided, including a 50-page report from the National Environmental Technology Applications Center (NETAC, 1993b) on the effectiveness of PRP in a mesocosm field study, a report from GMS Technologies (1999) on the evaluation of the BioSok product in a microcosm study, and company reports of two field trials. A summary of these case studies is described as follows.

- Bioremediation Product Evaluation: Mesocosm Field Study PRP Formulation #1 (NETAC, 1993b): A 21-day mesocosm study was conducted in a Petrol Rem testing facility to evaluate the performance a PRP product. Three treatments were tested in three equal size tanks (10 x 3 x 4 ft^3), where a gallon of fresh diesel fuel was added to each tank, and water from a natural source was pumped through continuously to simulate a flowing fresh water stream. The three treatments were 1) absorbent control (two one-pound sleeves of polypropylene absorbent), 2) no treatment control, 3) BioBoom + BioSok + PRP powder. GC/MS was used for hydrocarbon analysis, and the ratio of C17/pristane used as the measure of biodegradation effectiveness. The study concluded that PRP significantly stimulated the degradation of the hydrocarbon slick as compared to the absorbent and no treatment controls. Evidence of biodegradation was observed based on the slight decrease in C17/pristine ratio in the PRP tank. However, the report also pointed out that the PRP mode of action for the enhanced biodegradation (whether due to the added nutrients or beeswax material) was still unclear. One deficiency of this study was the lack of treatment replicates (only three tanks were used to test the three treatments). An inspection of the GC/MS data shown in the report revealed that the disappearance of all hydrocarbons appeared to take place at the same rate, suggesting that biodegradation may not have been the primary cause for the disappearance of the hydrocarbons. Support for this conclusion comes from a 3-D histogram of the data, from which it was very difficult to read concentrations.

- Evaluation of the BioSok product for boat bilge treatment: This study was conducted to test the effect of three treatments (untreated control, mini-BioSok with encapsulated oil-degrading *Bacillus sp*., and mini-BioSok without addition of bacteria) on remediation of bilge water using diesel fuel and bench-scale Bilge Model Reactors. Although this study was not conducted under field conditions, it is mentioned here because the test did help to understand the role of microorganisms in PRP. The results showed that the overall oil removal efficiencies were 71%, 84%, and 77% on average in four weeks for the cases of natural biodegradation, PRP without microbes, and PRP with encapsulation of microbes, respectively. A statistical analysis (Student's T-test) indicated that there was a significant difference between both PRP formulations and the no treatment control ($p < 0.10$), but no significant difference between the two PRP formulations ($p > 0.10$). However, since the treatments were not replicated, the statistical analysis was flawed since such an analysis requires replicate treatment units in order to calculate experimental error. Even if the results were statistically significant, the question remains if the incremental 13% benefit from using PRP justifies the cost of treatment compared to natural attenuation. The study further demonstrated that PRP relied on indigenous bacteria, not encapsulated ones, to perform the oil biodegradation.

- Field applications of PRP: Petrol Rem provided two real-world case studies. In both cases, only visual observation was used to monitor the performance of PRP treatment. In one case study, a barge carrying 4,000 metric tons of fuel oil sank in January 1998, resulting in fouling beaches and a mangrove along the Persian Gulf in Abu Dhabi. PRP was tested on site and visual observations (10 pictures enclosed in the report) found that the treated sections of the contaminated beach and mangrove showed marked improvement compared to untreated areas. Another case involved heavy oil spilled into a lagoon in Mexico in 1994. PRP was used to treat the water surface and the shore. After 26 days, it was estimated that approximately 75% of the original oil had been degraded based on visual examination of oil coverage on the lagoon surface (6 pictures enclosed in the report). However, use of visual observations with no attendant chemical analysis is not a reliable or scientifically sound method of assessing the treatability of a site by any technology.

3.2.2.7 Verde Environmental, Inc.

<u>*Product(s)*</u>

Verde Environmental, Inc. is the manufacturer of Micro-Blaze®, another oil bioremediation product listed on the NCP Product Schedule. Micro-Blaze is a liquid formulation of several microbial strains (*Bacillus* spores), surfactants, and nutrients designed to metabolize organics and hydrocarbons in soil and water (http://www.epa.gov/oilspill). Its commercial name is also called *Micro-Blaze Emergency Liquid Spill Control*. When in use, Micro-Blaze should be diluted with water to a certain percentage as determined by the type of contamination and applied through normal spray devices.

Case studies

Information on over a dozen full-scale applications of Micro-Blaze was provided in the *Handbook for Suggested Uses and Applications of Micro-Blaze Emergency Liquid Spill Control*, with one to a few pages of description for each case. The applications include hydrocarbon spill control, remediation of leaking underground petroleum storage tanks, wastewater treatment, and cleanup of firefighting training fields and food preparation sites. Two case studies that are relevant to this document are summarized as follows.

- Spill responses in a refinery plant: One anonymous major refinery plant frequently uses Micro-Blaze to cleanup its petroleum spills. For example, Micro-Blaze was used to remediate a crude oil spill in a tank-field in June 1997. TPH levels dropped from the low thousands to low hundreds of mg/kg in approximately eight weeks. In another case, a diesel spill in a pipe conduit occurred in November 1997. After the free product was pumped out, Micro-Blaze was used to finish the cleanup. In approximately four weeks, TPH concentrations dropped from a range of 25,000 - 57,000 mg/kg to a range of 600 - 9,900 mg/kg. Again, no controls were reported nor were treatments replicated.

- Gasoline spill clean up: A major gasoline spill (approximate 6000 gals) occurred in Conroe, Texas, on January 27, 1994, resulting in the contamination of the city sewage system. The wastewater treatment plant was shut down in the early morning and the gasoline-contaminated wastewater was diverted to a holding pond. Micro-Blaze Emergency Liquid Spill Control was sprayed to the basins and the lagoon in the wastewater treatment plant. Within ten minutes, the fumes disappeared. The wastewater treatment plant was able to operate at half capacity in the early afternoon and went back to normal by midnight. No analytical data, however, were presented in this case to demonstrate the effectiveness of Micro-Blaze as well as to explain the mode of action for this product. It is known that bioremediation is too slow acting to account for such a quick a hydrocarbon spill. It is likely that, since the spill was gasoline, most if not all of the treatment occurred as a result of volatilization.

3.2.2.8 WMI International, Inc.

Product(s)

WMI International's major oil bioremediation product is WMI-2000. This product is also listed on the NCP Product Schedule as a microbial additive. WMI-2000 is a dry power that contains microbial cultures specifically selected for remediation of petroleum products and other contaminants. The product may be applied as a dry powder or activated in water for 2 hours and applied to the surface of oil spills in water, pits, or ponds. It is also recommended by the manufacturer to apply nutrients with WMI-2000 microbes and maintain nitrogen and phosphorus concentrations in the treated water at 5-20 mg/L and 1-5 mg/L, respectively.

Case studies

Information on several pilot and full-scale applications of WMI-2000 was provided, which includes brief descriptions of three case studies and a company report with detailed analytical results on bioremediation of bilge water. A summary of two of these case studies is described as follows.

- Treatment of a hydrocarbon-contaminated storm water storage pond: WMI-2000 was used to clean up a hydrocarbon contaminated storm water storage pond for a major U.S. railroad. The pond was aerated with WMI Fine Bubble Diffusers and inoculated with WMI-2000 microbes. Visual observation was used to monitor the treatment performance. The results were illustrated using a series of pictures, which showed the reduction of oil coverage and the improvement of water clarity from Day 1 through Day 7 of the treatment. Again, since this study relied on visual observations for measurement of effectiveness, results must be discounted.

- Bioremediation of hydrocarbon contaminated water in ballast and bilge tanks: MWI-2000 was used to treat the ballast tank of a ship that was contaminated with coatings of heavy hydrocarbons, especially on the interior walls. During the first week of the treatment, 36 lbs. of WMI-2000 and 100 lbs of commercial fertilizer (12-24-0) were added in the ballast tank with a capacity of 179,000 gallons. Another 8 lbs of WMI-2000 each week was applied between the 2nd and 4th weeks. Forced air aeration of the bilge water was also applied continuously as part of the treatment. Analytical results using GC-FID showed that during the six weeks of the treatment, the concentrations of the various hydrocarbons were reduced from a range of 0.2- 4.3 mg/L to ND or 0.2 mg/L (detection limit), although no data was reported about the oil adhered to the interior walls of the vessel.

-

▪

Table 3.2 Summary of materials submitted by participating companies

Manufacturer or Vendor	List of submitted material
Enviro-Zyme, Inc.	• Product line cards: description, direction and MSDS about all Enviro-Zyme Products (BR, C, COMP, DOC, EZ, GT, L, LGD, M, N, O, P, POUCH, R, SEP, T) ▪ A copy of "BR Composite" that includes: ▪ Detail information about BR (primary ingredients, pH range, nutrient requirement, etc.) ▪ General considerations and summaries of application for the use of bioaugmentation products for assisting the cleanup of oil spills or soil contamination. ▪ Brief description of four case studies on the use of BR ▪ A journal paper promoting the use of bioaugmentation: Jensen, R.A. (1996) Bioremediation using bioaugmentation, *Environmental Technology*, 11/12, 1996
Forrester Environmental Technologies Corp. (FET Group)	• Information about BioCATalyst --a biostimulation product from botanical extraction. ▪ FET Group technical bulletins about BioCATalyst ▪ BOD5 test results from BSC (Biocatalyst Solution Company) ▪ MSDS ▪ Letter of acceptance from The Bureau of Petroleum Storage System, Department of Environmental Protection, Florida, for injection type of aquifer remediation ▪ Brief description of two case studies on the use of BioCATalyst with the attachment of hydrocarbon analysis reports
Garner Environmental Service, Inc.	• A catalog on Petro-Clean microbial products that includes: ▪ Petro-Clean products facts ▪ Brief description of four case studies ▪ Direction for use of Petro-Clean

	▪ MSDS
Industrial Wastewater Solutions Corp.	• A detailed letter of qualifications through email, which describes: ▪ Company background and its product IOS-500 ▪ A list of field applications and treatment results ▪ Areas of applications • A CD-ROM containing further information about IOS-500: ▪ Introduction of IOS-500 ▪ Brief descriptions of three case studies ▪ Letters of acceptance/acknowledgement from customers ▪ Pictures from the field ▪ Letter of recognition of non-pathogenicity for IOS-500 from Health Care Service of Alameda County, CA
Medina Agriculture Products Co., Inc.	• Brochures regarding Bioremediation Division of the company, its products and services. • A more detailed Medina Bioremediation Catalog, which includes: ▪ Procedure for using bioremediation products and an example of a land farming project ▪ Bioremediation compatibility testing ▪ Product guide ▪ List of support service and price quotes ▪ Domestic customer list ▪ MSDS • Summaries of three case studies on the effects of Medina Soil Activator • Detailed abstracts of two company reports on a lab treatability study and a field trial on Medina bioremediation products. • Two technical journal articles that involved the use of Medina bioremediation products:

	▪ Shafer, R. (1992). "Bioremediation of a California Land Site: a cost-effective way to treat oil contamination." Our California Environment, winter 1992. ▪ Shafer, R. (1991). "Cleanup old problems: Bioremediation of diesel contaminated soil and tundra." Land and Water, Nov/Dec, 1991
PetrolRem, Inc	• A PRP portfolio, including a PRP Bioboom-Biosock Product Video CD • Reports on PRP from governmental agencies and Petrol Rem's partners ▪ Review worksheet on PRP by U.S. Coast Guard's Alternate Response Tool Evaluation System (ARTES) program ▪ National Environmental Technology Applications Corporation (NETAC) (1993b). "Bioremediation Product Evaluation: Mesocosm Field Study PRP Formulation #1." ▪ GMS Report (1999). "Evaluation of the BioSok® product for boat bilge treatment and the reduction of non-point pollution," a research report by GMS Technologies to USEPA under EPA contract No. 68-D-98-138 ▪ Larry Lawson (1999). Excerpt from the Report to GMS Technologies on Commercialization Assessment of Improved Bio-Sok by Foresight Science & Technology under contract to the EPA • Company reports on two case studies ▪ Mexico field study results ▪ Abu Dhabi field test results with a letter of recognition by the customer
Verde Environmental, Inc.	• Three introduction and training videos ▪ Micro-Blaze microbial products for use in wastewater/septic systems ▪ Micro-Blaze emergency liquid spill control (training video) ▪ Micro-Blaze Out Microbial fire fighting agent (training video) • Handbook for suggested uses and applications, which include several documents and case histories, as well as regulatory agency acceptance letters • Acceptance letters from the states of Florida and Ohio that have been received since the manual was put together • A manual of technical papers, which include bioremediation background

	information and several biodegradability studies • A manual of toxicity, bioremediation tests, and chemical analysis, all of which was required by the EPA for Micro-Blaze to be placed on the EPA NCP product Schedule as a bioremediation agent • Various promotional flyers.
WMI International, Inc.	• Summary of three case studies on the effects of WMI-2000 microbial products • A company report: Bio-Remediation of Hydrocarbon Contaminated water in Ballast and Bilge Tanks, which include ▪ Treatment schedule in ballast tanks ▪ Hydrocarbon analysis report ▪ MSDS of WMI-2000 ▪ Toxicity test of WMI-2000 ▪ Letters from EPA and Department of Health & Human Services

3.2.3 Review of vendor reports

As shown in the previous section, the amount and quality of the submitted information 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). However, most of these cases involved treating relatively small-scale petroleum hydrocarbon contamination in somewhat confined environments (e.g., lagoons, land treatment units, ships, etc). The bioremediation applications were used as either a primary response strategy or a secondary polishing step after conventional mechanical cleanup options had been applied to remove free oil products. Oil and hydrocarbon contamination were observed to be reduced based on TPH analysis and visual observations. The submitted materials seem to suggest that these bioremediation products have satisfied many customers and have been able to meet regulatory requirements for their clients.

- 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. Among the ten bioremediation products described in the vendors' reports, six were bioaugmentation agents that contain hydrocarbon degraders (i.e., BR, Petro-Clean, IOS-500, Medina Hydrocarbon D-Grader, Micro-Blaze, and WMI-2000) and four were biostimulation agents containing either enzymes or nutrients but no active hydrocarbon degraders (i.e., BioCATalyst, Medina Microbial Activator, Bio-D Nutrients, and PRP). All of these microbial products contained nutrients and surfactants or required applying with nutrient products. In most of the reported field applications (bioaugmentation or biostimulation), certain types of oxygen amendment were used as well, such as tilling of contaminated soil or forced air aeration of polluted water. Therefore, no conclusion can be made solely based on these reports in regard to the determination of the limiting factors (microbes, nutrients or enzymes) for oil biodegradation, although in some cases there was some evidence that microbial amendments did not enhance oil biodegradation better than biostimulation (GMS Report, 1999; Shafer, 1992).

- 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. As described in Chapter 2, evidence for the effectiveness of oil bioremediation in terms of oil biodegradation 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. To obtain evidence of increased rate of disappearance, a set-aside untreated area or a control should be used, which has similar physical and biological conditions as the treated site. However, no controls were used in most of the reported case studies. Although oil spill responders prefer not to set aside any oiled untreated sites, it is difficult to assess the true impact of a treatment without control or set-aside areas. To effectively distinguish biodegradation from abiotic loss, specific oil components or analytes should be analyzed occasionally using GC/MS techniques. These analytes should be normalized to a conserved biomarker, such as hopanes and/or alkyl-substituted chrysenes. Again, except for the report on the mesocosm test of PRP (NETAC, 1993b), none of these cases used GC/MS analysis. In most of the cases, oil/hydrocarbon concentrations were evaluated by simple TPH analysis. For others, visual observation was the only method of monitoring the treatment performance. Since all TPH techniques are severely affected by spatial heterogeneity, it is essential that a well-thought out sampling plan be designed according to valid statistical principles involving randomization and replication of treatments in order to ensure that monitored results reflect reality in such a highly heterogeneous environment,. Unfortunately, very little if any statistical analyses were conducted to support the conclusions and claims made by the vendors and writers of those reports. Considering current regulatory requirements and the cost of the hydrocarbon analysis, it may not be realistic for oil spill responders to conduct detailed GC/MS analyses during oil spill bioremediation applications (except perhaps an occasional sample). However, in order to provide more convincing evidence of product effectiveness, the oil bioremediation industry can and should do more to acquire the highest quality data possible within budget restraints, such as following sound statistical principles of experimental design and adopting a well-designed sampling plan.

3.3 Bioremedial Approaches for Controlling Petroleum Hydrocarbons in Stormwater and Bilge Water.

One of the objectives of this review was to evaluate and assess the use of bioremediation technology for the cleanup of hydrocarbon contamination from storm water discharges and boat bilges. After a thorough search of the literature, however, little information was available on the field experience pertaining to the use of bioremediation agents for treating petroleum hydrocarbons in stormwater and bilge water. Therefore, the potential of using bioremediation products in these non-point sources can only be briefly discussed as follows.

3.3.1 Bioremediation of hydrocarbon contamination from storm water discharges

It is estimated that about 0.12 metric tons of petroleum hydrocarbons are released into the world's water through urban runoff every year, which make up approximately 4% of oil input into the oceans (NAS, 1985). Urban runoff is also the leading source of impairments to surveyed estuaries according to recent *National Water Quality Inventory* reports (U.S. EPA; 1996).

Technologies that are currently used as Best Management Practices (BMPs) for controlling hydrocarbons in stormwater include oil and grease trap devices, wet detention ponds, wetland systems (phytoremediation), and filter systems (Botts *et al*. 1996, Shutes *et al*., 1997, Schueler, 1987, U.S. EPA, 1997). Trap devices for removing oil and grease from stormwater include various mechanical oil-water separators, oil and grease skimmers, and water quality inlets. The skimmer is often used at the outlet of a sediment basin. Water quality inlets consist of a series of chambers or basins that remove sediment, screen debris, and separate free oil from storm water. Wet detention ponds maintain a permanent pool of water in addition to temporarily holding stormwater, and they provide both quality and quantity control of storm water. Hydrocarbons in stormwater are degraded through natural attenuation. Constructed or restored wetlands are also effective means of controlling low-level hydrocarbon contamination, which use green plants and their associated rhizosphere microorganisms to degrade and contain pollutants. Nix *et al*. (1994) investigated the performance of a wetland system for treating storm runoff containing diesel fuel and found that 96% of the total extractable hydrocarbons were removed after only five hours retention. Another bioremedial technology for treating stormwater is compost stormwater filters (CSF). This innovative system removes contaminants from stormwater by allowing water to pass through layers of specially tailored compost. A CSF can typically remove over 90% of all solids and 85% of oil and grease (U.S. EPA, 1997).

Although the approach of adding biostimulation and/or bioaugmentation agents has not been selected as BMPs for the treatment of hydrocarbon contamination in stormwater, limited information gathered suggests that application of bioremediation agents could be a promising approach, especially used in conjunction with other stormwater countermeasures, such as wet detention ponds. For example, BR (Enviro-Zyme, Inc) was used to assist in treating an industrial runoff containing oil and grease in an aeration lagoon during winter seasons, when the existing treatment system could not meet the regulatory standard (see Section 3.2.2.1). WMI-2000 also treated hydrocarbon-contaminated stormwater in a wet detention pond, but this reported success was based on visual observation (see Section 3.2.2.8). However, further field tests are still required to evaluate the effectiveness of this strategy, particularly to determine whether addition of microbial cultures is necessary or effective in enhancing oil biodegradation in stormwater.

3.3.2 Bioremedial approaches for treating bilge oil

Bilge oil discharge is another major source of petroleum contamination for the world's navigable waters. It is estimated that bilge oil comprises 9% of oil input into the oceans (NAS, 1985). Currently international regulation does not allow ships to discharge bilge water containing more than 15 mg/L of oil. Some of the BMPs for reducing the amount of oil from boat bilges entering marinas and surface waters include: promoting the installation and use of fuel/air separators on air vents; avoiding overfilling fuel tanks, maintaining proper engine performance and routinely checking for fuel leaks; promoting the use of materials that capture or digest oil in bilge water; extracting used oil from absorption pads if possible; and prohibiting the use of detergents and emulsifiers on fuel spills (U.S. EPA, 2001).

Commonly used methods for *in-situ* treatment of bilge water include oil water separators, such as centrifuges, for large vessels and absorption devices, also called "bilge socks" or "bilge pads", for smaller recreational boats. Some of these products are also combined with bioremedial processes. For example, a "bio-mechanical" oil water separator, trademarked as PetroLiminator, was developed recently by Ensolve Biosystems, Inc. (Raleigh, NC). This device is a non-pressurized three-stage vessel: Stage 1 allows for initial separation of heavy or pure oil. The retained emulsified oil is then biodegraded in a separate biofilm reactor at Stage 2. Stage 3 consists of a final clarifier for solids removal, and the effluent is then discharged. PetroLiminator has been tested in sea trials (MarineLog.com, 2001) and has been type-approved by U.S. Coast Guard (USCG) and the International Maritime Organization (IMO).

Another bioremedial method for treating bilge oil is the use of absorption pads that may also contain bioremediation agent(s). BioSok is one such product. According to the manufacturer, PetrolRem, each BioSok, which measures 9 inches in length by 3 inches in diameter, contains eight ounces of PRP. It is purported to absorb up to one pound of contaminant and degrade much more oil over time. As mentioned previously, BioSok achieved about 80% diesel removal in a microcosm study, slightly better than the performance of natural attenuation. However, no scientifically verifiable field data in regard to the rate and capacity of oil biodegradation for this product is available.

Considering the simplicity of use and environmental friendliness of the processes, products like BioSok could be a promising solution for bilge water treatment. However, this type of product also faces tough competition from other absorption devices. Tests conducted by consumer-oriented organizations often emphasize the oil absorption capacity and the firmness of the oil binding (Boat U.S. Foundation, 2001; Costa, 2000), which normally is not the strength of the bioremedial type of bilge pads. Therefore, more work needs to be done in terms of both market improvement and technical demonstration of these bioremedial sorbents for them to reach their potential in the area of bilge oil treatment.

4 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. There are two main approaches to oil spill bioremediation: 1) *bioaugmentation,* in which oil-degrading microorganisms are added to supplement the existing microbial population, and 2) *biostimulation*, in which the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth-limiting cosubstrates and/or habitat alteration. Bioremediation agents are also classified as bioaugmentation and biostimulation agents based on these two main bioremedial approaches. Since the objective of this document was to conduct a thorough assessment of bioremediation products by a comprehensive review of their actual use in real world cases, it was hoped that documented field experiences would be able to provide the more convincing argument for the effectiveness of bioremediation technology. Literature reviewed included peer-reviewed journal articles, company reports, government reports, and actual reports by cleanup contractors engaged in the response to spills in inland, estuarine, and marine environments. The key findings of this literature review are summarized bellow:

- 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. However, many case studies have demonstrated that bioremediation can also be used as a primary response strategy, especially for the cleanup of environmentally sensitive areas that are not amenable to conventional cleanup techniques and/or low-level petroleum hydrocarbon contamination.

- 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 cases scientifically deficient.

- 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. In other words, background nutrient concentrations at the contaminated site should be one of the primary determining factors in the decision to apply nutrients, and biostimulation might not always be necessary if sufficient nutrients are naturally present at a spill site to supply non-limiting concentrations to the degrading populations. However, effects of nutrients are also highly site-specific. For example, the availability of oxygen rather than nutrients is often the limiting factor in wetland environments, where addition of nutrient products has not been successful in enhancing oil biodegradation (although it has been successful in accelerating the restoration of the affected plant biomass to an abundant and rich recovery).

- Different nutrient products have shown variable effectiveness, depending on oil properties, the nature of the nutrient products, and the characteristics of the contaminated environments. Based on limited field trials, it appears that slow-release fertilizers may be an excellent choice if the nutrient release rates are balanced against physical loss rates; water-soluble fertilizers may be more cost-effective in low-energy shorelines and fine-grained sediments where water transport is limited; and oleophilic fertilizers may be more suitable for use on hard, rocky shorelines, although further research is still required to confirm this suggestion. In general, commercial oleophilic nutrient products have not shown clear advantages over common agricultural fertilizers in stimulating oil biodegradation.

- 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. Bioremediation agents may also be effective for the treatment of bilge water, although, due to the lack of any systematic investigation into its effectiveness, it is still uncertain whether this approach could compete with other existing technologies. Further field tests are needed to provide stronger evidence on the potential of this strategy.

- 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 for the environments described in this report, especially estuaries, experiments based on sound scientific principles are needed. Unfortunately, resources for field-testing commercial bioremediation agents are scarce, and field studies are extremely expensive to carry out. That's why it is best to rely on laboratory microcosm or mesocosm studies to provide needed data to support this technology. When spills occur and the on-scene coordinator in conjunction with the Regional Response Team decides to implement commercial bioremediation for cleanup, they should try to set aside control areas if at all possible to allow a more effective evaluation of treatment success. If this practice is carried out, a true advancement in knowledge will be possible.

- If there is any hope for advancement of commercial bioremediation, especially estuaries, experiments based on sound scientific principles are needed. Unfortunately, due to the extreme resource intensiveness of field studies, the benefit accruing to testing one bioremediation agent is only applicable to the one product being tested, not to the overall science of bioremediation. Testing products in the field is not within the purview of the federal government unless such a test has the potential of advancing science in terms of general microbiological and engineering principles.

5 References

Aldrett, S., Bonner, J.S., McDonalds, T.J., Mills, M.A., Autenrieth, R.L. (1997) Degradation of crude oil enhanced by commercial microbial cultures. *Proceedings of 1997 International Oil Spill Conference*. American Petroleum Institute, Washington DC, pp995-996.

Alleman, B.C., and E.A. Foote. 1997. Evaluation of Amendments for Enhancing Microbial Activity in Soils from Site 18 at MCAGCC Twentynine Palms, California. Battelle, Columbus, OH. Performing Organization Report Number D.O. 1795. Sponsoring Agency Report Number TCN 96-026. Feb. 7, 1997.

Athey, P., Reeder, D., Lukin, J., McKendrick, J., Con, J.S. (2001) *Tundra Treatment Guidelines: A Manual for treating Oil and Hazardous Substance Spills to Tundra*. Alaska Department of Environmental Conservation, Juneau, Alaska.

Atlas, R.M. (1995) Bioremediation of petroleum pollutants. *International Biodeterioration & Biodegradation*, 317-327.

Atlas, R. M. (ed.)(1984) *Petroleum Microbiology*. Macmillan Publishing Company, New York.

Atlas, R.M. (1981) Microbial degradation of petroleum hydrocarbons: An environmental perspective. *Microbiol. Rev*. **45**, 180-209.

Atlas, R.M. (1977) Stimulated petroleum biodegradation. *Crit. Rev. Microbiol*., **5**, 371-386.

Atlas, R.M. and Bartha R. (1992) Hydrocarbon biodegradation and oil spill bioremediation. In K.C. Marshall (ed.), *Advances in Microbial Ecology, Vol. 12*, Plenum Press, NY, pp287-338.

Atlas, R.M. and Bartha, R. (1973) . Effects of some commercial oil herders, dispersants and bacterial inocula on biodegradation of oil in seawater. In Ahearn and Meyers (Eds.), *The Microbial Degradation of Oil Pollutants*, Publication No. LSU-SG-73-01, Louisiana State University, Baton Rouge, LA, pp283-289.

Azarowicz, R.M. (1973) Microbial degradation of petroleum. *US Patent* 3,769,164.

Blenkinsopp, S., Sergy, G., Wang, Z., Fingas, M. F., Foght, J., Westlake, D. W. S. (1995) Oil spill bioremediation agents: Canadian efficiency test protocols. *Proceedings of 1995 International Oil Spill Conference*, American Petroleum Institute, Washington, DC, pp91–96.

Boat U.S. Foundation (2001) Pillow talk: go soak your bilge, Foundation finding report #34, http://www.boatus.com/foundation/findings/oil_removal_products.htm

Botts, J., Allard, L., and Wheeler, J. (1996) Structural Best Management Practices for Storm Water Pollution Control at Industrial Facilities, *Proceedings of Watershed' 96 Conference*, Water Environment Federation, pp216-219.

Bragg, J.R., Prince, R.C., Harner, E.J., and Atlas, R.M. (1994) Effectiveness of bioremediation for the *Exxon Valdez* oil spill. *Nature,* **368**, 413-418.

Bragg, J.R., Prince, R.C., Wilkinson, J.B., and Atlas, R.M. (1992) *Bioremediation for Shoreline Cleanup Following the 1989 Alaskan Oil Spill*. Exxon Co., USA. Houston.

Burns, K.A., Codi, S., Duke N.C. (2000) Gladstone, Australia field studies: weathering and degradation of hydrocarbons in oiled mangrove and salt marsh sediments with and without the application of an experimental bioremediation protocol. *Marine Pollution Bulletin*, **41**, 392-402.

Costa, J.E. (2000) A review of the performance of bilge socks proposed for use in Buzzards Bay recreational boats in response to a request for proposals issued by the Buzzards Bay action Committee and the Town of Dartmouth, Massachusetts, Buzzards Bay Project, National Estuary Program. http://www.buzzardsbay.org/bilgesockwebreport.pdf

Douglas, G.S., Prince, R.C., Butler, E.L., and Steinhauer, W.G. (1994) The use of internal chemical indicators in petroleum and refined products to evaluate the extent of biodegradation. *In* R.E. Hinchee, B.C. Alleman, R.E. Hoeppel, and R.N. Miller (eds.), *Hydrocarbon Bioremediation*, Lewis Publishers, Inc., Boca Raton, FL. pp. 219-236.

Foght, J.M. and Westlake, D.W.S. (1987) Biodegradation of hydrocarbons in freshwater. In: Vandermeulen and Hrudey (Ed), *Oil in Freshwater: Chemistry, Biology, Countermeasure Technology.* Pergamon Press, New York, pp217-230.

Forsyth, J.V., Tsao, Y.M., Blem, R.D. (1995) Bioremediation: when is augmentation needed? In Hinchee, R.E. *et al.* (eds) *Bioaugmentation for Site Remediation*. Battelle Press, Columbus, OH, pp1-14.

Garcia-Blanco, S., Motelab, M., Venosa, A.D., Suidan, M.T., Lee, K., King, D.W. (2001) Restoration of the oil-contaminated Saint Lawrence River shoreline: Bioremediation and Phytoremediation. *Proceedings of 2001 International Oil Spill Conference*. American Petroleum Institute, Washington DC, pp. 303-308.

Glaser, J.A. (1994) Engineering approaches using bioremediation to treat crude oil-contaminated shoreline following the *Exxon Valdez* Accident in Alaska. In P.E. Flathman *et al*. (eds.), *Bioremediation: Field Experience*, Lewis Publisher, Boca Raton, pp81-103.

Glaser, J.A., Venosa, A.D., Opatken, E.J. (1991) Development and evaluation of application techniques for delivery of nutrients to contaminated shoreline in Prince William Sound. *Proceedings of 1991 International Oil Spill Conference*. American Petroleum Institute, Washington, DC, pp559-562.

GMS Technologies (1999) *Evaluation of the BioSok® product for boat bilge treatment and the reduction of non-point pollution*, a research report by GMS Technologies to USEPA under EPA contract No. 68-D-98-138.

Goldstein, R.M., Mallory, L.M., Alexander, M. (1985) Reasons for possible failure of inoculation to enhance biodegradation. *Applied and Environmental Microbiology*, **50**, 977-983.

Hoff, R. (1993). Bioremediation: An overview of its development and use for oil spill cleanup. *Marine Pollution Bulletin* **26** (9), 476-481.

Hoff, R. (1991) *A Summary of Bioremediation Application Observed at Marine Oil Spill*. Report HMRB 91-2, National Oceanic and Atmospheric Administration, Seattle, Washington.

Hozumi, T., Tsutsumi, H. and Kono, M. (2000) Bioremediation on the shore after an oil spill from the *Nakhodka* in the Sea of Japan. I. Chemistry and characteristics of the heavy oil loaded on the *Nakhodka* and biodegradation tests on oil by a bioremediation agent with microbial cultures in the laboratory. *Marine Pollution Bulletin*, **40**, 308-314.

Jobson, A.M., Cook, F.D., and Westlake, D.W.S. (1974) Effect of amendments on the microbial utilization of oil applied to soil. *Appl. Microbiol*. **27**, 166-171.

Jorgenson, M.T. and Cater, T.C. (1996) Minimizing ecological damage during cleanup of terrestrial and wetland oil spills. In: Cheremisinoff (Ed), *Storage Tanks.* Gulf Publishing Company, Houston, TX, pp257-293.

Ladousse, A. and Tramier, B. (1991) Results of 12 years of research in spilled oil bioremediation: Inipol EAP 22, *Proceedings of 1991 Oil Spill Conference*. American Petroleum Institute, Washington, DC, pp577-581.

Lawson, L (1999) Excerpt from the Report to GMS Technologies on Commercialization Assessment of Improved Bio-Sok by Foresight Science & Technology under contract to the EPA.

Le Floch, S., Merlin, F.X., Guillerme, M., Dalmazzone, C., and Le Corre, P. (1999) A field experimentation on bioremediation: Bioren. *Environmental Technology*, **20**, 897-907.

Le Floch, S., Merlin, F.X., Guillerme, M., Tozzolino, P., Ballerini, D., Dalmazzone, C., and Lundh, T. (1997) Bioren: recent experiment on oil polluted shoreline in temperate climate. In: *In-Situ and On-Site Bioremediation*: Volume 4, Battelle Press, Columbus, OH, pp. 411-417.

Leahy, J.G.; Colwell, R.R. (1990) Microbial Degradation of hydrocarbons in the environment. *Microbial Reviews*, **53**(3), 305-315.

Lee, K., and Levy, E.M. (1989) Enhancement of the natural biodegradation of condensate and crude oil on beaches of Atlantic Canada. *Proceedings of 1989 Oil Spill Conference*. American Petroleum Institute, Washington, DC, pp 479-486.

Lee, K., and Levy, E.M. (1987) Enhanced biodegradation of a light crude oil in sandy beaches. *Proceedings of 1987 Oil Spill Conference*. American Petroleum Institute, Washington, DC, pp411-416.

Lee, K., and Trembley, G. H. (1993). Bioremediation: Application of slow-release fertilizers on low energy shorelines. *Proceedings of the 1993 Oil Spill Conference*, American Petroleum Institute, Washington, D. C., pp449-454.

Lee, K., Tremblay, G.H., and Gauthier, J., Cobanli, S.E., Griffin, M. (1997) Bioaugmentation and biostimulation: a paradox between laboratory and field results. *Proceedings of 1997 International Oil Spill Conference*. American Petroleum Institute, Washington DC, pp697-705.

Lee, K., Tremblay, G.H. and Cobanli, S.E. (1995) Bioremediation of oiled beach sediments: Assessment of inorganic and organic fertilizers. *Proceedings of 1995 International Oil Spill Conference*. American Petroleum Institute, Washington, DC, pp107-113.

Lin, Q., Mendelssohn, I. A., Henry, C. B., Roberts, P.O., Walsh, M.M., Overton, E. B., Portier, R. J. (1999) Effects of bioremediation agents on oil degradation in mineral and sandy salt marsh sediments. *Environmental Technology*, **20**, 825-837.

Linn, R.R. (1971) Method for soil restoration. *US Patent* 3,769,164.

Madden, P.C.; Hinton, S.M. Lee, M.D. (1991) *Bioremediation of a Contaminated Beach on Prall's Island*, Project report from Exxon Research & Engineering Company and Dupont Environmental Remediation Services, Inc.

MarineLog.com (2001) “Bilge water treatment system completes trials”, Newupdates, July 19, http://www.marinelog.com/DOCS/NEWS/MMJul19a.html

Mauro, G. and Wynne, III, B.J. (1990) *Mega Borg* oil spill: an open water bioremediation test. Texas General Land Office, Austin, Texas.

Mearns, A. J. (1997) Cleaning oiled shores: putting bioremediation to the test. *Spill Science & Technology Bulletin*, **4**(4), 209-217.

Mearns, A. J., Roques, P., Henry C.B.Jr. (1993) Measuring efficacy of bioremediation of oil spills: monitoring, observations, and lessons from the Apex oil spill experience. *Proceedings of 1993 International Oil Spill Conference*. American Petroleum Institute, Washington DC, pp335-344.

Mearns, A. (1991) Observation of an Oil Spill Bioremediation Activity in Galveston Bay, Texas. NOAA Technical Memorandum NOS OMA 57, National Oceanic and Atmospheric Administration, Seattle, Washington.

Mendelssohn, I. A., Lin, Q., Debusschere, K., Henry, C. B., Overton, E. B., Portier, R. J., Walsh, M. M., Penland, S., Rabalais, N. N. (1995) The development of bioremediation for spill cleanup in coastal wetlands: Product impacts and bioremediation potential. *Proceedings of the 1995 Oil Spill Conference*, American Petroleum Institute, Washington, DC, pp97-100.

Mohan, R.R., Byrd, G.H., Nixon, J., and Bucker, E.R. (1975) Use of microorganisms to disperse and degrade oil spills. *US Patent* 3,871,957.

National Academy of Sciences (1985) *Oil in the Sea: Inputs, Fates and Effects*, National Academy Press, Washington DC.

National Environmental Technology Application Center (1993a) *Evaluation Methods Manual: Oil Spill Response Bioremediation Agents*. University of Pittsburgh Applied Research Center, Pittsburgh, PA.

National Environmental Technology Application Center (1993b) *Bioremediation Product Evaluation: Mesocosm Field Study PRP Formulation #1*. University of Pittsburgh Applied Research Center, Pittsburgh, PA.

Nichols, W.J. (2001) The U.S. Environmental Protect Agency: National Oil and Hazardous Substances Pollution Contingency Plan, Subpart J Product Schedule (40 CFR 300.900). *Proceedings of 2001 International Oil Spill Conference*. American Petroleum Institute, Washington DC, pp1479-1483.

Nix, P.G., Stecko, J.P., and Hamilton, S.H. (1994) A constructed wetland for the treatment of stormwater contaminated by diesel fuel. 17th Arctic and Marine Oil Spill Program Technical Seminar, VI, 439-464, Environment Canada.

NOAA (1992) *Shoreline Countermeasure Manual*, National Oceanic & Atmospheric Administration, Seattle, Washington.

Office of Technology Assessment (1991), *Bioremediation of Marine Oil Spills: An Analysis of Oil Spill Response Technologies*, OTA-BP-O-70, Washington, DC.

Office of Technology Assessment (1990), *Coping With An Oiled Sea: An Analysis of Oil Spill Response Technologies*, OTA-BP-O-63, Washington, DC.

Oil Spill Intelligence Report (2000) *20th International Oil Spill Control Directory*, Cutter Information Corp., Arlington, MA.

Oudot, J., Merlin, F.X., and Pinvidic, P. (1998) Weathering rates of oil components in a bioremediation experiment in estuarine sediments. *Marine Environmental Research*, **45**(2), 113-125.

Prince, R.C. (1993) Petroleum spill bioremediation in marine environments. *Critical Rev. Microbiol*. **19**, 217-242.

Prince, R. C., Bare, R. E., Garrett, R. M., Grossmann, M. J., Haith, C. E., Keim, L. G., Lee, K., Holtom, G. J., Lambert, P., Sergy, G. A., Owens, E. H., and Guenette, C. C. (1999) Bioremediation of a marine oil spill in the Arctic. In B.C. Alleman & A. Leeson (eds) : *In-Situ*

Bioremediation of Petroleum Hydrocarbon and Other Organic Compounds, Battelle Press, Columbus, OH, pp. 227-232.

Prince, R. C., Clark, J.R., Lindstrom, J.E., Butler, E.L., Brown, E.J., Winter, G., Grossman, M.J., Parrish, P.R., Bare, R.E., Braddock, J.F., Steinhauer, W.G., Douglas, G.S., Kennedy, J.M., Barter, P.J., Bragg, J.R., Harner, E.J., and Atlas, R. M. (1994) Bioremediation of the Exxon Valdez oil spill: monitoring safety and efficacy. In: R.E. Hinchee *et al.*(Eds.), *Hydrocarbon Bioremediation*. Lewis Publishers, Boca Raton, Florida, pp107-124.

Pritchard, P.H, Mueller, J.G, Rogers, J.C., Kremer, F.V. and Glaser, J.A (1992) Oil spill bioremediation: experiences, lessons and results from the Exxon Valdez oil spill Alaska. *Biodegradation* **3**, 109-132.

Pritchard, P.H., Costa, C.F., and Suit, L. (1991) *Alaska Oil Spill Bioremediation Project*. EPA/ 600/9-91/046a&b, Office of Research and Development, U.S. EPA, Gulf Breeze, FL.

Rosenberg, E. and Ron, E.Z (1996) Bioremediation of petroleum contamination, In R.L. Crawford and D.L. Crawford (Eds.), *Bioremediation: principles and Applications*, Cambridge University Press, UK, 100-124.

Rosenberg, E., Lagmann, R., Kushmaro, A., Taube., R., Adler, R., and Ron, E.Z. (1992) Petroleum bioremediation—a multiphase problem. *Biodegradation*, **3**, 337-350

Safferman, S.I. (1991) Selection of nutrients to enhance biodegradation for the remediation of oil spilled on beaches. *Proceedings of 1991 International Oil Spill Conference.* American Petroleum Institute, Washington DC, pp571-576.

Santas, R. and Santas, P. (2000) Effects of wave action on the bioremediation of crude oil saturated hydrocarbons. *Marine Pollution Bullrtin*, **40**(5) 434-439.

Schueler, T.R., (1987) *Controlling Urban Runoff: A Practical Manual for Planning and Designing Urban BMPs*. Prepared for Washington Metropolitan Water Resources Planning Board, Washington, D.C.

Sendstad, E. (1980) Accelerated biodegradation of crude oil on Arctic shorelines, *Proceedings of the Third Arctic and Marine Oil Spill Program Technical Seminar*, Environment Canada, Ottawa, Canada.

Shafer, R. (1992) Bioremediation of a California Land Site: a cost-effective way to treat oil contamination. *Our California Environment*, winter 1992.

Shafer, R. (1991) Cleanup old problems: Bioremediation of diesel contaminated soil and tundra. *Land and Water*, Nov/Dec, 1991.

Shin, W.,S., Tate, P.T., Jackson, W.A., Pardue, J.H. (1999) Bioremediation of an experimental oil spill in a salt marsh. *In* Means and Hinchee (Eds): *Wetlands and Remediation: An international Conference*. Battelle Press, Columbus, OH, pp.33-40.

Shutes, R.B.E., Revitt, D.M., Mungur, A.S., and Scholes, L.N.L. (1997) The design of wetland systems for the treatment of urban run off. *Wat. Sci. Tech*., **35**(5), 19-25.

Simon, M., Autenrieth, R.L., McDonald, T.J., Bonner, J.S.(1999) Evaluation of bioaugmentation for remediation of petroleum in a wetland. *Proceedings of 1999 International Oil Spill Conference*. American Petroleum Institute, Washington DC.

Spies, R.B., Rice, S.D., Wolfe, D.A., Wright, B.A. (1996) The effect of the Exxon Valdez oil spill on Alaskan coastal environment, *Proceedings of the 1993 Exxon Valdez Oil Spill Symposium*, American Fisheries Society, Bethesda, MD, pp1-16.

Sveum, P., and Ramstad, S. (1995) Bioremediation of oil-contaminated shorelines with organic and inorganic nutrients. In Hinchee, R.E. *et al.* (eds) *Applied Bioremediation of Petroleum Hydrocarbons*. Battelle Press, Columbus, OH, pp201-217.

Sveum, P., Faksness, L.G., and Ramstad (1994) Bioremediation and of oil-contaminated shorelines: the role of carbon in fertilizers. *In* R.E. Hinchee, B.C. Alleman, R.E. Hoeppel, and R.N. Miller (eds.), *Hydrocarbon Bioremediation*, Lewis Publishers, Inc., Boca Raton, FL. pp. 163-174.

Swannell, R. P. J., Mitchell, D., Lethbridge, G., Jones, D., Heath, D., Hagley, M., Jones, M., Petch, S., Milne, R., Croxford, R., and Lee, K. (1999a) The use of bioremediation to treat an oiled shoreline following the Sea Empress Incident. In B.C. Alleman & A. Leeson (eds) : *In-Situ Bioremediation of Petroleum Hydrocarbon and Other Organic Compounds*, Battelle Press, Columbus, OH, pp. 239-244.

Swannell, R. P. J., Mitchell, D., Lethbridge, G., Jones, D., Heath, D., Hagley, M., Jones, M., Petch, S., Milne, R., Croxford, R., and Lee, K. (1999b) A field demonstration of the efficacy of bioremediation to treat oiled shorelines following the Sea Empress Incident. *Environmental Technology*. **20**: 863-873.

Swannell, R.P.J., Lee, K., and McDonagh, M. (1996) Field evaluations of marine oil spill bioremediation. *Microbiological Reviews*, **60**(2), 342-365.

Swannell, R.P.J., Croft, B.C., Grant, A.L., and Lee, K. (1995) Evaluation of bioremediation agent in beach microcosms, *Spill Science & Technology Bulletin*, **2**(2/3) 151-159.

Townsend, R.T., Bonner, J.S., Autenrieth, R.L.(1999) The effect of bioremediation on microbial populations in an oil-contaminated coastal wetland. *Proceedings of 1999 International Oil Spill Conference*. American Petroleum Institute, Washington DC.

Tsutsumi, H., Kono, M., Takai, K., Manabe, T. (2000) Bioremediation on the shore after an oil spill from the *Nakhodka* in the Sea of Japan. III. Field test of a bioremediation agent with microbiological cultures for the treatment of an oil spill. *Marine Pollution Bulletin*, **40**, 320-324.

U.S. Army (1999) *Biodegradation of POL-Contaminated Washrack Sludge*, Public Works Technical Bulletin 420-49-27, 31 March 1999, Department of the Army, U.S. Army Corps of Engineers, Washington DC

U.S. EPA (2002) Spill *NCP Product Schedule*, http://www.epa.gov/oilspill

U.S. EPA (2000) *The Quality of Our Nation's Waters: A summary of the National Water Quality Inventory: 1998 Report to Congress*. EPA841-S-00-001, Office of Water, U.S. Environmental Protection Agency.

U.S. EPA (1999) *Understanding oil spills and oil spill response*, EPA 540-K-99-007, Office of Emergency and Remedial Response, U.S. Environmental Protection Agency.

U.S. EPA (1997) *Innovative Use of Compost: Bioremediation and Pollution Prevention*. EPA530-F-97-042, Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency.

U.S. EPA (1996) *A Series of Fact Sheets on Nonpoint Source (NPS) Pollution*. EPA841-F-96-004, Office of Water, U.S. Environmental Protection Agency.

U.S. EPA (1993) *Guidance Specifying Management Measures for Sources of Nonpoint Pollution in Coastal Waters*, EPA 840-B-92-002**,** Office of Water, U.S. Environmental Protection Agency.

U.S. EPA (1992) *Bioremediation Case Studies: An Analysis of Vendor Supplied Data*, EPA/600/R-92/043, Office of Research and Development, U.S. Environmental Protection Agency.

Venosa, A.D. (1998) Oil spill bioremediation on coastal shorelines: a critique. In: S.K. Sikdar & R.I. Irvine(Eds.), *Bioremediation: Principles and Practice*. Vol. III. *Bioremediation Technologies.* Technomic, Lancaster, PA, pp259-301.

Venosa, A. D., Lee, K., Suidan, M. T., Garcia-Blanco, S., Cobanli, S., Moteleb, M., Haines, J.R., Tremblay, G., and Hazelwood, M. (2002) Bioremediation and biorestoration of a crude oil-contaminated freshwater wetland on the St. Lawrence River. *Bioremediation Journal*, **6** (3).

Venosa, A. D., Suidan, M. T., Wrenn, B. A., Strohmeier, K. L., Haines, J. R., Eberhart, B. L, King, D.W., and Holder, E. (1996) Bioremediation of experimental oil spill on the shoreline of Delaware Bay. *Environmental Science and Technology*, **30**, 1764-1775.

Venosa, A.D., Suidan, M.T., Haines, J.R., Wrenn, B.A., Strohmeier, K.L., Eberhart, J.R., Kadkhodayan, M., Holder, E., King, D., Anderson, B. (1995) Field bioremediation study: spilled crude oil on Bowler Beach, Delaware. In Hinchee, R.E. *et al.* (eds) *Bioaugmentation for Site Remediation*. Battelle Press, Columbus, OH, pp49-56.

Venosa, A.D., Haines, J.R., Eberhart, B.L. (1997) Screening of bacterial products for their crude oil biodegradation effectiveness. In Sheehan (ed) *Methods in Biotechnology, Vol.2: Bioremediation Protocols*. Humana Press Inc., Totowa, NJ, pp47-57.

Venosa, A.D., Kadkhodayan, M., King, D., Wrenn, B.A., Haines, J.R., Herrington, T., Strohmeier, K.L., and Suidan, M.T. (1993) Testing the efficacy of oil spill bioremediation products, *Proceedings of 1993 International Oil Spill Conference*. American Petroleum Institute, Washington DC, pp487-493.

Venosa, A.D., Haines, J.R., and Allen, D.M. (1992) Efficacy of commercial inocula in enhancing biodegradation of crude oil contaminating a Prince William Sound beach. *J. Ind. Microbiol*., **10**, 1-11.

Venosa, A.D., Haines, J.R., Nisamaneepong, W., Govind, R., Pradhan, S., Siddique, B. (1991) Protocol for testing bioremediation products against weathered Alaskan crude oil. *Proceedings of 1991 International Oil Spill Conference*. American Petroleum Institute, Washington DC, pp563-570.

Venosa, A.D., Haines, J.R., Nisamaneepong, W., Govind, R., Pradhan, S., Siddique, B. (1990) *Protocol for Testing Bioremediation Products against Weathered Alaskan Crude Oil.* EPA/600/D-90/208, Risk Reduction Engineering Laboratory, U.S. EPA, Cincinnati, OH.

Wickham, D. E (1995) US Patent #5.531.898

WMI Report (2001) Bio-Remediation of Hydrocarbon Contaminated Water in Ballast and Bilge Tanks, WMI International, INC., Huston, TX.

Zhu, X., Venosa, A.D., Suidan, M.T., and Lee, K. (2001) Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands, Report under a contract with Office of Research and Development, U.S. Environmental Protection Agency. http://www.epa.gov/oilspill/pdfs/bioremed.pdf

Zobell, C. E. (1946) Action of microorganisms on hydrocarbons. *Bacteriol. Rev*. **10**, 1-49.

Zwick, T.C., Foote, E.A., Pollack, A.J., Boone J. L., Alleman, B.C., Hoeppel, R.E., Bowling L. (1997) Effects of nutrient addition during bioventing of fuel contaminated soils in an arid environment. In: *In-Situ and On-Site Bioremediation*: Volume 1, Battelle Press, Columbus, OH, pp. 403-409.

Appendix A: Initial Letter Calling for Information

Dear Madam/Sir:

I am a research associate with the University of Cincinnati's Department of Civil and Environmental Engineering, working on a contract with the U.S. Environmental Protection Agency's Office of Research and Development. I am conducting an in-depth literature review on the efficacy of commercial bioremediation products for cleaning up oil-contaminated environments, with special emphasis on estuaries. The objective of this project is to conduct a thorough assessment of the use and effectiveness of commercial bioremediation products by reviewing actual field cases where bioremediation has been used.

Based on information from EPA's Oil Program Center, the National Contingency Plan Product Schedule, and the Oil Spill Intelligence Report (Cutter Information Corp.), it is my understanding that your company has been involved in producing and marketing bioremediation products for oil spill cleanup. Since we intend to make our review as inclusive and fair as possible, the field experience of your company on bioremediation agents is extremely valuable to us. Therefore, I would like to obtain some technical information and experience in regard to your bioremediation product(s). The information that is of particular interest to me includes the following:

- Principle ingredients of your bioremediation products (without your divulging confidential business information).
- Technical publications regarding the effectiveness of your bioremediation products in actual case studies.
- Client response and contact information.

The purpose of this letter is to see whether your company is willing to participate in this inquiry. Please understand that your participation in this endeavor is strictly voluntary. If you wish to respond, please drop me a quick note via e-mail, and I will contact you via telephone at your convenience and will provide you more detailed information about my request. If you do not wish to participate, please also let me know so that I may strike you from my list.

Due to the time constraint of this project, I would be most appreciative if you would reply within two weeks. Thank you for taking the time to consider this request, and I am very hopeful to receive a positive reply from you.

Sincerely yours,

Xueqing Zhu, Ph.D., P.E.
Department of Civil and Environmental Engineering
University of Cincinnati
Cincinnati, OH 45221-0071
Tel: 513-556-3638, Fax: 513-556-2599
E-mail: zhuxi@email.uc.edu

Appendix B: Follow-up Letter to Participating Companies

Dear Madam/Sir:

Thank you for your willingness to participate in this information collection effort as a part of EPA's investigation of the efficacy of bioremediation products. I am writing to you to provide more information about this project and what I need from you. The objective of this project is to conduct an in-depth literature review on the efficacy of commercial bioremediation products for cleaning up oil-contaminated environments. The scope of this review is limited to the use of bioremediation agents (i.e., microbial additives and nutrient additives) for cleanup of surface oil spill (inland, estuarine, and marine environments, but not groundwater). The performance of other oil spill control agents and approaches may be included only as a point of reference and comparison. The information that is of particular interest to me is listed as follows:

(1) Principle ingredients of your bioremediation products.

Without your divulging confidential business information, can you tell me whether your products include any living organisms (bacteria, fungi, etc.), nutrients, enzymes, exogenous hydrocarbons, sorbents, surfactants, or anything else that characterizes your product and its primary mechanism of action?

(2) Efficacy for oil spill cleanup in the field.

Do you have any technical publications, client reports, third-party reports, and/or company reports regarding the effectiveness of your bioremediation products in actual case studies? Information that would be most helpful includes:

- Type of spilled oil and extent of contamination;
- Effectiveness data on hydrocarbon destruction or removal;
- Analytical methods used to support your conclusions;
- Detailed sampling plan design;
- Any statistical analysis done;
- Environment in which the product was used;
- Anything else you would deem important to my investigation.

(3) Client contact information.

If you don't mind, it would be most helpful if you could reveal the names, addresses, and telephone numbers or email addresses of your clients so that I could contact them personally to interview them for their view on how well bioremediation worked in their instance.

Our final report to EPA is due in September. I would be most appreciative if you would provide the above information by the end of March. I will be happy to send a copy of our completed report when it is finished, peer-reviewed, and cleared by the Agency, should you desire one. Thanks again for your help. If you need any additional information about this request, please do not hesitate to contact me.

Sincerely yours,

Xueqing Zhu, Ph.D., P.E.
Department of Civil and Environmental Engineering
University of Cincinnati
Cincinnati, OH 45221-0071
Tel: 513-556-3638, Fax: 513-556-2599
E-mail: zhuxi@email.uc.edu