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BIOREMEDIATION: A COUNTERMEASURE FOR MARINE OIL SPILLS

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Introduction

Among the many countermeasures that are available for use during marine oil spills, bioremediation has received increasing attention during recent years. Background information on the technique for the broad group of people who are involved with spill response activities, including both on-scene responders as well as planners and researchers, is provided in this article. The Hazardous Materials Response and Assessment Division of the United States National Oceanic and Atmospheric Administration (NOAA) has been involved in several spills where bioremediation has been considered or used. These incidents are summarized and a critique of the effectiveness of each is provided. The lessons learned from associated monitoring programs are discussed.

Background

Biodegradation versus bioremediation

Biodegradation is the natural process whereby bacteria or other microorganisms alter and break down organic molecules into other substances, such as fatty acids and carbon dioxide. *Bioremediation* is the act of adding fertilizers or other materials to contaminated environments, such as oil spill sites, to accelerate the natural biodegradation process (U.S. Congress, 1991). Bioremediation is also used in other applications, including sewage treatment, terrestrial oil spills, and experimentally for hazardous wastes.

Three main types of bioremediation technologies are currently being developed or used for treatment of oil spills: addition of nutrients to oiled

This issue is dedicated to bioremediation of oil spills. Bioremediation has recieved a good deal of attention in the past two years. Rebecca Hoff, of NOAA, describes what bioremediation is and reviews a number of recent spill incidents. She concludes that bioremediation is largely an experimental technique for open water spills and may not be successful in many applications. Shoreline and land application of fertilizer, however, can result in measurable removal of oil.

Canada

Environment Canada   Environnement Canada

shorelines, addition of microbes to oiled shorelines, and addition of nutrients and/or microbes to open water oil slicks. Since all of these technologies attempt to accelerate biodegradation, the processes of biodegradation of oil are summarized; some of the potential uses of this technology are discussed, including specific instances where bioremediation has been applied at oil spills; and guidelines for evaluating and monitoring bioremediation applications are presented.

How does biodegradation work?

Biodegradation is one of the main ways in which spilled oil is weathered. It occurs in most environments, but at varying rates, depending on localized environmental conditions and on the composition of the oil (for example, heavier oils are more resistant to biodegradation than lighter oils; Atlas, 1975). Among the many environmental factors that will affect biodegradation rates, oxygen, nutrients, and temperature are probably the most important (Atlas, 1981; DeFlaun and Mayer, 1983).

Simply adding oil to an environment will stimulate growth of indigenous microbes, since the oil provides increased amounts of carbon, the microbes' food source (Lee and Levy, 1991). Several researchers have documented a lag period before indigenous microbial communities begin to degrade oil (NOAA, 1980; Fusey and Oudot, 1984). This may be due to the fact that oil is initially toxic to microbial organisms, and the most toxic fractions must be weathered before microbes can grow, a time period of several days to several weeks (Lee and Levy, 1989b).

The primary processes of microbial degradation are aerobic (requiring oxygen), though anaerobic degradation may occur at very low rates. Low-energy, sheltered environments probably have the lowest rates of biodegradation, especially in subsurface sediments. Oil in anaerobic sediments in marshes or other environments may degrade very little, with oil persisting in some cases for several years (Delaune *et al.*, 1980; Atlas, 1981; Lee and Levy, 1991). High-energy environments (more exposed, with greater impact from waves) usually show rapid biodegradation, in part because of physical weathering, but also because wave action supplies oxygen and nutrients to the microbial communities, facilitating biodegradation (Lee and Levy, 1989a).

Microbial populations that undergo rapid growth in the presence of spilled oil may become limited by inadequate amounts of nutrients, such as nitrogen and phosphorus. Field tests on biodegradation of a waxy crude oil (Terra Nova crude) in sandy beaches found that fertilizer addition was effective in accelerating biodegradation in areas that were heavily oiled, but less so in areas that were lightly oiled (little acceleration was measured in these areas). This was due to the fact that unassisted biodegradation occurred very rapidly in the lightly oiled areas (Lee and Levy, 1991). Nutrients are more likely to be limiting to the biodegradation on oiled shorelines or oil slicks, than for degradation of suspended oil particles in the water column (Atlas, 1981).

At extremely high salinities, biodegradation is inhibited (Ward and Brock, 1978), but this is not likely to be a problem in the normal range of salinities usually encountered in marine and coastal environments (Lee and Levy, 1989a).

Types of Bioremediation

1. Nutrient addition

The theory behind bioremediation by nutrient addition is simple: microbes already living on an impacted shoreline have a sudden new source of food–carbon compounds in the spilled oil. After the initial toxicity of the oil decreases (through evaporation of the volatile compounds) and after indigenous species of hydrocarbon-degrading microbes become acclimated, they begin to break down the oil, and their population grows. At this point, the sudden increase in numbers of microbes may deplete existing supplies of nutrients and this may limit further growth of the microbial population. The microbial population can continue to increase with added nutrients, and degrade oil at a faster overall rate, than it could without the supplemental nutrients.

Numerous laboratory studies on nutrient enhancement of oil degradation by naturally occurring microbes have concluded that this technique is promising for use on oiled shorelines (NOAA, 1978; Atlas, 1981; Lee and Levy, 1987; 1989a). Field experiments have also been conducted, but these have not always corroborated the laboratory results (Fusey and Oudot, 1984; Lee and Levy, 1991).

Conducting field tests of bioremediation has proved difficult for several reasons. For one, it is not easy to quantitatively measure biodegradation outside of the laboratory. Showing a statistically significant difference in biodegradation rates between control areas and fertilized areas has proved especially difficult. This is partly because oil on shorelines tends to be distributed in a very uneven manner in sediments, resulting in high variability in data from chemical analyses of sediment samples. The variability in biodegradation rates measured in the field is also reflective of environmental variability at the site, including parameters such as temperature or substrate type (Prince *et al.*, 1990; DuPont, 1991; Pritchard *et al.*, 1991).

The potential advantages of any bioremediation technique must be balanced against possible detrimental environmental effects, including introduction of contaminants, toxicity to aquatic organisms, and physical impacts. Some fertilizer products, whose primary use is in a terrestrial setting, may contain trace elements as micro-nutrients (e.g., copper or mercury) that would be introduced into an aquatic environment with potentially much more significant toxicological effects (Mearns, 1991). Others may produce by-products such as ammonia and/or nitrates that are toxic to aquatic organisms at certain concentrations (U.S. EPA, 1989). Intertidal organisms that are directly exposed during application of the undiluted nutrient solution may be adversely impacted. In addition, physical disturbance from the application process and from monitoring will have some impacts on the shoreline, especially in sensitive environments such as marshes.

Nutrient addition is still experimental in marine environments; therefore, any application should include a monitoring program to determine whether the desired objectives are being met, and whether potentially detrimental impacts have been minimized to an acceptable level. Following are summaries of different types of nutrient formulations and application techniques that have been used in recent bioremediation applications.

Types of nutrient formulations. Nutrient addition can include a variety of application techniques as well as numerous commercial products (usually fertilizers), some that have been developed specifically for use on oil spills, and others that have been adapted from agricultural or domestic use. These products can be grouped into three basic categories: soluble inorganic nutrients, oleophilic formulations, and slow release formulations.

Soluble inorganic nutrients. Inorganic nutrients include a wide variety of water-soluble garden or agricultural fertilizers that can be mixed with sea water and sprayed on shorelines. These fertilizers can be formulated with different ratios of nitrogen and phosphorus and usually include small quantities of trace elements. Some advantages of inorganic nutrients are that they are readily available, inexpensive, and usually consist of compounds with well known properties. However, since these formulations are water-soluble, they may be washed off the shoreline by tidal action, requiring frequent, repeated applications. There may also be some direct toxicity to plants or animals in the intertidal zone that are directly impacted during the application process.

Oleophilic formulations were developed to solve the problem of solutions washing off rocks or beaches, and to provide nutrients at the oil water interface, where bacteria will be metabolizing the oil. Oleophilic (literally, "oil-loving") products are chemically "sticky" and adhere to oil on rocks or other substrates. These formulations are designed to remain at the oil-water interface and to be readily accessible to oil-degrading microbes. In Prince William Sound, Alaska, the oleophilic product Inipol EAP 22 was applied extensively to oiled shorelines and was investigated in several monitoring studies.

Inipol contains oleic acid (a source of carbon), urea (a source of nitrogen), tri(laureth-4)-phosphate (a surfactant), and 2-butoxy-ethanol (another surfactant) (Pritchard *et al.*, 1991). Since addition of oil alone will stimulate bacterial growth, the presence of oleic acid in the fertilizer complicates evaluating the effectiveness of oleophilic formulations such as Inipol. *Do these products appear to work better because the microbes are eating the carbon in the product instead of the spilled oil?* Lee and Levy (1989b) concluded that adding an oleophilic product to a low-energy beach contaminated with crude oil was ineffective as a bioremediation agent because the microbes were preferentially eating the organic components of the product instead of the oil.

Several scientists have argued that Inipol appears to be effective because it is acting primarily as a chemical surfactant rather than as a bioremediation agent. Surfactants, such as the ones in Inipol, are found in cleaning agents and dispersants. Inipol contains approximately 10% 2-butoxy ethanol, a common ingredient in household cleaning agents, and also one of the ingredients in the dispersant Corexit 9527 (Exxon, 1989a; 1989b). Critics have argued that some of the immediate visual effects noted during field observations using oleophilic products (such as changes in colour, or disappearance of surface oil; Chianelli *et al.*, 1991) may result from their surfactant properties, rather than from stimulated biodegradation.

Several components of Inipol are toxic to humans and other organisms at certain concentrations. These include 2-butoxy-ethanol and urea, which produces ammonia when it comes in contact with water. 2-butoxy-ethanol is toxic to mammals, especially in the first 48 hours after application. Effects on humans include eye and skin irritation, and damaged blood cells with repeated exposure. This requires that special safety precautions be taken for workers who handle Inipol, such as wearing clothing (rubber boots and aprons) or respirators if exposure to fumes or dust is likely (Exxon, 1989b).

Slow-release formulations are designed to release quantities of nutrients over a longer period of time, and to remain in the area where they are applied. They include various products with mixes of nitrogen, phosphorus and other compounds, packaged in dissolvable capsules or briquettes. These formulations are designed to release small quantities of nutrients slowly over a period of time. While briquettes may move about on the beaches with tidal action, granules will usually lodge among pebbles and cobbles and remain in the intertidal zone. In this way, the concentration of soluble nutrients is controlled at low levels and release of nutrients to the subsurface may be facilitated as granules work their way down into sediments.

Several types of slow-release products were tested in Prince William Sound in 1989 by EPA, and one granule product, Customblen, was subsequently applied extensively on shorelines (Glaser *et al.*, 1991; Pritchard *et al.*, 1991). Customblen contains nutrients (ammonium nitrate, calcium phosphate, and ammonium phosphates) encased in a polymerized vegetable oil (Prince *et al.*, 1990). Assuming that the pellets remain in the intertidal zone, Customblen does not need to be applied as frequently as liquid formulations. Some possible disadvantages include the possibility that pellets may wash away or lodge at the high tide zone on high-energy beaches. Concentrations of pellets higher than the recommended application could collect in one location (such as a tidal pool) and create concentrations of ammonia that could be toxic to aquatic organisms.

Nutrient applications

Exxon Valdez
March 1989 - 1991

In March 1989, approximately 300 miles of shoreline in Prince William Sound were oiled with North Slope crude oil from the *Exxon Valdez* spill (Pritchard and Costa, 1991). In the early summer, following preliminary results from a bioremediation test program conducted by the EPA's Alaska Oil Spill Bioremediation Project, the Alaska Regional Response Team (RRT)* approved the use of bioremediation with nutrients to treat oiled shorelines. A number of constraints were placed on the application of nutrients, including a restriction to areas that were well-flushed, and a prohibition

* Regional Response Teams are composed of representatives from various government agencies involved with natural resources. The RRT has the authority to approve certain activities at oil spills, including use of dispersants and bioremediation.

from applying nutrients in sensitive areas such as those near anadromous fish streams. The decision to apply bioremediation to specific shorelines was made on an area-by-area basis. In 1990, continued use of bioremediation as a shoreline treatment was approved with the requirement that a monitoring program be conducted to evaluate the effectiveness and safety of the bioremediation applications (Lindstrom *et al.*, 1991; U.S. Congress, 1991; Prince *et al.*, 1990; Pritchard *et al.*, 1991).

The studies conducted in 1989 and 1990 in Prince William Sound were comprehensive and investigated the effectiveness of different types of fertilizers at several sites, including replicate test and control plots. Monitoring also included sampling and analysis of various water quality parameters and toxicity testing (Prince *et al.*, 1990; Pritchard *et al.*, 1991).

In 1989, one of two treated test plots in Passage Cove (treated with water-soluble fertilizer applied with sprinklers) showed statistically significant differences in oil residue weight when compared with the control site. The second test site (treated with Inipol and Customblen) did not show a significant difference in oil residue weight compared with the control site. (Measures of oil residue weight varied at all sites by up to two orders of magnitude). Microbial counts showed no significant differences in numbers of microbes between treated and control plots. However, significant differences were found between numbers of bacteria at oiled sites versus unoiled sites, demonstrating that the presence of oil alone will stimulate microbial growth (Glaser *et al.*, 1991; Pritchard *et al.*, 1991).

At a second study conducted in 1989 at Snug Harbour, measurements of oil residue weight over time were highly variable among all plots, including control plots. (Values ranged over an order of magnitude.) Decreasing trends in oil residue weight were found at all plots, including the control plot. No data showing statistical comparisons were presented, but there do not appear to be strong differences between control and treated plots in oil residue weight loss over time. Gas chromatographic analyses were conducted, showing degradation rates that appeared to be higher at treated sites (Pritchard *et al.*, 1990; 1991).

The studies conducted in 1990 encountered the same problem as those in 1989 with highly variable distributions of oil in sediments. In addition, remaining oil ranged from fairly fresh to very weathered among the test sites. This resulted in such variability in oil residue weight, that detecting differences in a quantitative analysis would have been impossible without approximately an order of magnitude more samples (Prince *et al.*, 1990).

Additional chemical analyses used the marker compound hopane to track biodegradation of the more weathered oil from 1990. Samples of subsurface oil taken from three sites showed strong (statistically significant) increases in biodegradation rates at one site, and less strong results from the other two sites. These results were an encouraging demonstration of the effectiveness of bioremediation for use on subsurface oil (Exxon, 1992).

There are several reasons why the results from both years were variable: background rates of biodegradation were found to be "surprisingly high" at control plots; second, there could be strong differences in local environmental conditions that either favour or inhibit biodegradation at each individual site. Further, a process of declining returns would be expected in 1990, since most of the remaining oil was weathered, and thus more resistant to biodegradation. These studies can be interpreted as showing that *some fertilized sites* clearly demonstrated the effectiveness of nutrient addition in stimulating biodegradation, while at other sites less stimulation was achieved.

Many descriptions of the bioremediation applications following *Exxon Valdez* have reported its success in degrading oil on Prince William Sound shorelines (Chianelli *et al.*, 1991; Crawford, 1990; Pritchard and Costa, 1991). Careful evaluation of the data from the monitoring studies leads to a more cautious conclusion, however. Statistically significant increases in degradation rates compared with controls were found at some sites during both 1989 and 1990. However, these increases were not found at all test sites, and increases over the entire experiment were not statistically different from controls (Prince *et al.*, 1990; Pritchard *et al.*, 1991).

Bioassays conducted using oyster and mussel larvae showed some acute toxicity, while bioassays using mysids showed no acute effects. These were conducted with water samples from beaches after treatment with both Inipol and Customblen (Sanders and Gray, 1989; Prince *et al.*, 1990; Pritchard *et al.*, 1991). No chronic toxicity tests were conducted, nor were analyses made for sediment toxicity or direct toxicity of Inipol to intertidal organisms.

Water quality measurements found no enrichment of nutrients in waters surrounding bioremediated beaches, or evidence of increased algal growth (Prince *et al.*, 1990).

Prall's Island, New Jersey
June 1990 - December 1990

In January 1990, a pipeline broke at the Exxon Bayway refinery in Linden, New Jersey. Fuel oil was spilled into the Arthur Kill waterway, contaminating a beach on the Prall's Island bird sanctuary. Most of the oil was removed by physical means, but these efforts were halted in March 1990, partly to avoid effects on migrating birds using the area. Exxon Research and Engineering received permission to conduct a bioremediation experiment on part of the beach with remaining oil (DuPont, 1991).

The experiment used a slow-release fertilizer (Customblen) placed in two shallow trenches dug in the intertidal zone. In an attempt to get around the usual high variability in distribution of oil on the beach, bags of beach substrate containing known concentrations of oil were buried in each test plot. Samples from each of these bags were measured for total petroleum hydrocarbons at the end of the experiment to compare rates of biodegradation (DuPont, 1991). Microbial counts made on beach samples taken before fertilization showed high background levels of microbes in the test area. Background levels of nitrogen and phosphorus were also high when measured prior to the beginning of the experiment (DuPont, 1991).

The overall results showed that biodegradation was occurring at the site, though differences between the control plot and the fertilized plot were not statistically significant. This was, in part, due to the high variability in the levels of total petroleum hydrocarbons (TPH) measured in soils and sampling bags from all plots, including the control plot.

A major problem with the study was the fact that the control plot was apparently influenced by nutrients that leached from the treated plots. This compromising of the control may have obscured any differences between it and the treated plots. Additionally, the fact that high levels of microbes were measured in the substrate before the fertilizer treatment began may be an indication that background levels of biodegradation were naturally high at this site. This is not surprising since Prall's Island has been chronically affected by oil spills in the past, and indigenous microbial populations may be well adapted to the presence of hydrocarbons. The relatively high levels of nitrogen and phosphorus measured at the site before fertilizer application began suggest that nutrients may not be a limiting factor in this system.

Though no bioassays were conducted as part of this experiment, levels of ammonia in offshore and interstitial water (water in the pore spaces between sediment particles) were generally below levels that would be toxic to aquatic organisms (U.S. EPA, 1989). Levels of dissolved oxygen in offshore waters and in the interstitial waters of the test plots were monitored throughout the experiment. Ammonia levels were highest in the lower intertidal areas of the treated plots, ranging from 4 to 10 ppm, while levels at the control plot ranged from 0 to 2 ppm.

2. Microbial addition

Adding microbes to contaminated areas, also known as "seeding," is conducted with the aim of enhancing biodegradation of an oil-impacted area with selected strains of microbes that are known to be capable of degrading hydrocarbons. However, the effectiveness of this technique is not well supported in the scientific literature (Atlas, 1981). In fact, studies indicate that addition of microbes to an open environment may not increase biodegradation because "foreign" strains of bacteria are frequently out-competed by indigenous species, and thus disappear quickly from the microbial community (Lee and Levy, 1989b).

No strain of bacteria, whether indigenous or from an outside source, is likely to degrade oil actively until after the most toxic components of the oil have evaporated (Lee and Levy, 1987). Therefore, claims of "instant success" from products containing microbes should be regarded with scepticism. The argument is made that indigenous organisms will be killed by the oil, so new microbial species need to be added to begin the process of biodegradation. In fact, studies have found that most areas of the world contain some microbes that are capable of degrading oil, and that the microbes usually grow rapidly when they have acclimated to an oil spill (Lee and Levy, 1989a).

Currently, no genetically engineered microorganisms are being considered for use in bioremediation (U.S. Congress, 1991).

To date, few objective scientific studies have been conducted that have tested commercial products containing microbial formulations. The most comprehensive was conducted by Al Venosa's laboratory at the EPA Office of Research and Development in Cincinnati (Venosa and Haines, 1991; Venosa *et al.*, 1991a; 1991b). In brief, the lab study compared the biodegradation of weathered Prudhoe Bay crude oil using individual applications of eleven commercial microbial products with a nutrient-only control. Two products showed a statistically significant increase in biodegradation over the nutrient control. However, these products performed as well with sterilized (dead) microbes as with live microbes. Both of the two highest performers were then tested in a controlled, replicated field test in Alaska. In the field, no significant difference in oil residue weight or total resolvable alkanes** could be detected among the control plots, the fertilized plots, or the plots treated with microbial products.

Most microbial products either contain or recommend use of some type of nutrients, so concerns about potential toxicity also apply here. In addition, microbial products should be screened to detect potential human or animal pathogens. Other chemicals that are possibly part of microbial products (such as binders or surfactants), could also be toxic to aquatic organisms. The question of potential toxicity can be answered by conducting bioassays or other standard toxicity tests.

Microbial applications

Apex Barges, Texas
August 1990

A collision between three Apex barges and the tanker *Shnoussa* occurred on July 28, 1990, spilling approximately 2 650 000 L (700 000 gallons) of a partially refined oil into Galveston Bay. Shorelines and marshes along the northern shore of the Bay were contaminated by the oil approximately one week after the initial spill. The Texas Water Commission received approval from the Region 6 RRT to conduct a trial application of a microbial bioremediation product (Alpha BioSea) to a contaminated marsh (Mearns, 1991). Regional Response Team approval was given under certain guidelines, including that the application be done only in areas where mechanical recovery of oil was not feasible, and that a scientifically sound monitoring program be conducted. The Texas Water Commission carried out the monitoring program with consultation from NOAA and EPA representatives who also acted as on-site observers.

On August 5 the pre-mixed solution containing the microbial product and a nutrient mix was applied to the marsh by a high-pressure hose from a small boat. Samples of water and sediment were collected both before treatment and at approximately 24, 48, and 96 hours after the treatment. All samples were sent to an EPA laboratory for analysis (Nadeau *et al.*, 1991).

No noticeable differences between treated and untreated plots could be discerned in samples collected 48 hours after treatment. This was verified by data from chemical analyses of surface and bottom water samples. Gas chromatography tracked the indicator compounds C18/phytane and C17/pristane over time, comparing treated and control plots. Samples collected at 0, 24, 48, and 96 hours after treatment showed little trend over

** Alkanes are straight-chain, single bond hydrocarbon molecules that are more easily degraded than other compounds in oil, such as cyclic or aromatic compounds.

time, and no statistically significant differences in degradation rates were found between samples from treated and untreated sites (Nadeau *et al.*, 1991).

The chemistry results are not surprising for several reasons. First, since Galveston Bay is chronically affected by oil spills, one would expect that indigenous populations of bacteria would be well adapted for hydrocarbon degradation. Therefore, it is questionable whether microbial addition enhanced the biodegradation process at all in this type of environment. Second, the short period of the monitoring could probably not have measured any acceleration in biodegradation rates if this had, in fact, occurred, since biodegradation usually does not begin until several days or weeks after a spill. Third, there is no way to separate influences due to the microbial product from influences due to the fertilizer. Lastly, the feedstock oil involved in this incident is a form that is already partially degraded, perhaps making it a less promising candidate for bioremediation (Mearns, 1991; Nadeau, 1991).

The monitoring program experienced several problems which impeded the collection of useful information and affected the results of the experiment. These included poor control over application of the product, disturbance of the test areas by livestock and numerous human activities, too few samples collected, and a too-short period of sample collection after the application (Mearns, 1991; Nadeau, 1991).

Water samples collected after the application were tested for toxicity to silversides and mysids. Samples from a composite of two sites were acutely toxic to mysids. None of the samples were toxic to silversides. Additional concerns about potential toxic effects of trace elements in the nutrient mix were raised by Mearns (1991).

Seal Beach, California
November 1990

A well blowout offshore of Seal Beach, California occurred on October 31, 1990, releasing approximately 1500 L (400 gallons) of crude oil into the atmosphere, resulting in the oiling of approximately two to three acres of marsh grasses in the Seal Beach National Wildlife Refuge (U.S. Depart. of the Interior, 1990; U.S. EPA, 1991).

Bioremediation treatment with a microbial product plus fertilizer was undertaken one week after the blowout, followed by an application of fertilizer alone two weeks later. Treatment consisted of hand spraying of grass blades with a combination of a microbial product used in sewage treatment plants (INOC 8162) and a commercial fertilizer (Miracle Gro 30-6-6). Samples of unoiled, oiled and treated, and oiled and untreated grass were collected and analyzed by the EPA Environmental Research Laboratory in Gulf Breeze, Florida (U.S. EPA, 1991).

The results of a number of laboratory tests performed on samples taken from the marsh showed no differences between oiled and treated grasses and oiled grasses with no treatment. Measures of degradation included most probable number counts of bacteria and ^{14}C mineralization, a relative measure of biodegradation rate. In addition, a laboratory study was performed by EPA to compare the ability of the INOC product to degrade Prudhoe Bay crude oil with uninoculated (nutrient only) controls. Little or no difference was found in the amount of four indicator compounds between the flask containing the product and the control flask after 7 and 16 days of incubation. This indicates that the microbial product was not effective in accelerating biodegradation of oil under these controlled laboratory conditions (U.S. EPA, 1991).

The United States Fish and Wildlife Service has collected samples of plants and invertebrates and intends to analyze these tissues for presence of hydrocarbon compounds (Goodbred, 1991). These analyses have not yet been performed, and no other toxicity testing has been reported to date.

3. Open-water bioremediation

Studies from the early 1970s in laboratory and simulated large tank situations have investigated the use of addition of nutrients on open-water oil slicks (Atlas and Bartha, 1973). However, to date, no studies have evaluated use of bioremediation (microbial or nutrients) in an open ocean situation. From a research viewpoint, it is still unknown whether bioremediation would be effective on a recently spilled, open-water oil slick.

Biodegradation in the water is thought to occur at the water surface (Lee and Levy, 1989a). Therefore, any product or nutrient added would need to stay at this interface and follow the oil slick as it moves. For bioremediation to be successful on open water, the nutrients or microbes would have to remain with the oil slick for the time it takes microbes to become acclimated to the oil and begin biodegrading.

As in shoreline applications, the question has been: "*Do bioremediation products applied on open water actually act as dispersants or surfactants, and actually redistribute oil into the water column, rather than break down the oil through bioremediation?*" If this is the case, "*Should these products then be considered dispersants and not bioremediation agents?*"

The same concerns for potential toxicity that have been discussed for use of nutrient additions and microbes on shorelines also apply to open-water applications. The dilution factor is likely to be much greater on open water, however, and this is likely to lessen the risk from direct toxic effects. As with dispersants, monitoring will present very real difficulties, including formidable logistics for applications, measurements or observations, and the difficulty of collecting samples that will provide meaningful data.

Open-water applications

***Mega Borg*, Texas**
June 1990

The *Mega Borg* spill in 1990 is the only known use of a microbial addition to an open-water oil slick in the United States. The *Mega Borg* super tanker was transferring its cargo of Angolan crude oil approximately 60 nautical miles off the coast from Galveston, Texas, when an explosion caused a fire and subsequent discharge of oil. Oil was released continuously for nine days. The Region 6 RRT gave approval to the Texas Water Commission to conduct an experimental, open-water application of a microbial product to the slick. The microbial product was applied twice from a Coast Guard vessel, six and nine days after the initial explosion. Sampling was conducted from a Texas A&M University research vessel and included samples of surface water and subsurface samples from 1- and 9-m depths. Three of these samples were sent to the EPA Gulf Breeze lab for toxicity testing (Parrish and Albrecht, 1990; Research Planning Inc., 1991).

Several problems were encountered during the experiment, including interference by skimmers working in the same area where the first application was made, and logistical problems with the sampling vessel, resulting in no sample collection during the first application. A dispersant test was also conducted during this spill (Payne *et al.*, 1991), causing further competition for logistics platforms.

Results from the percentage of oil found in samples of mousse from surface water were inconclusive since no differences could be detected between samples collected before and after the bioremediation application. As stated by the report: "The high variability in these samples ... demonstrates the difficulty of obtaining comparative and consistently representative samples in the open ocean, and the unequal mixing of the oil on the water surface." (Texas General Land Office, 1990/p.10).

The Texas General Land Office relied heavily on visual observations made several hours after the application to evaluate the experiment (Texas General Land Office, 1990). Since it is unlikely that microbial activity could have begun this quickly after application it is likely that the observed visual changes in the appearance of the slick were caused by physical processes such as dispersion, or absorption of the oil by binder materials in the bioremediation product. This experiment demonstrates both the difficulties inherent in attempts to conduct open-water experiments and the inconclusive results that may result from open-water bioremediation.

Results of the EPA Gulf Breeze lab's acute (96-h) bioassays performed on silversides and mysids showed no acute effects, but the researchers questioned whether the samples were actually collected in an area affected by the oil slick, since no trace of oil was found in the samples (Parrish and Albrecht, 1990). The bioremediation product was not directly tested for toxicity.

Monitoring Recommendations

There is no single measure that will accurately measure effectiveness or toxicity of a bioremediation application. Most of the larger bioremediation monitoring programs that have been undertaken have used a combination of the techniques discussed, depending on their specific concerns and objectives (Prince *et al.*, 1990; DuPont, 1991; Pritchard *et al.*, 1991). For example, the *Exxon Valdez* studies measured several different parameters for toxicity, water quality, and effectiveness (Table 1). As a minimum, a monitoring plan at a bioremediation field test or application should include at least the following endpoints:

1) To measure effectiveness, track changes in indicator hydrocarbon compounds (such as C18/pristane, C17/phytane, or hopane) using gas chromatography/ mass spectroscopy (GC/MS). As a minimum, collect samples at the beginning and end of the application period at control and treated sites.

2) Conduct toxicity testing using bioassays to determine acute and/or chronic toxicity to aquatic organisms. Include sediment bioassays if the bioremediation compounds are likely to lodge in sediments. Sample at treated and control sites.

3) Monitor environmental impact on aquatic habitats through chemical analysis of sediments or water for potentially toxic compounds (such as heavy metals) that may be part of a bioremediation product. Collect samples at the beginning and end of the application period at control and treated sites.

Discussion

As a countermeasure, bioremediation is a useful addition to the toolbox of existing spill treatment technologies, including the option of no treatment. Bioremediation is promising because it enhances biodegradation, which occurs naturally in most areas where oil is spilled. In fact, in areas that are lightly oiled, natural, unassisted biodegradation may be the best "treatment." The key question to ask in these circumstances is:" *Can bioremediation offer an improvement over natural levels of biodegradation?*" Perhaps the most enlightening aspect of many of the field studies of bioremediation is that unassisted biodegradation occurs at rapid rates in many environments, and that the no-treatment option can be an appropriate "countermeasure" in many cases.

Of the three types of bioremediation discussed here, nutrient addition seems to hold the most immediate promise, especially for use in areas that would be adversely affected by physical or other removal methods. Environments where nutrient addition may play an important role in shoreline treatment include:

- sheltered shorelines that are heavily oiled, when physical methods are not feasible or have already been attempted;
- shorelines with subsurface oil that may degrade very slowly; and
- sensitive environments, especially marshes and wetlands.

Nutrient addition is less likely to yield effective results under the following circumstances:

- in environments that are already nutrient-rich; and
- for short-term, immediate response actions.

Microbial additions and open water bioremediation are still experimental technologies, and as such, their effectiveness still needs to be documented in real environments. Further, until screening mechanisms for commercial products are in place (such as those being developed by the U.S. EPA a considerable burden rests with the spill responder to evaluate unknown (and often proprietary) formulations. If data are available to show that microbial products contain no toxic elements, small-scale field trials provide an opportunity to test their effectiveness.

In conclusion, the nature of biodegradation and the variable results from field tests confirm that bioremediation is not an off-the-shelf technique that can be applied to oiled shorelines with the expectation of success in all

**Table 1 Bioremediation Case Histories
Treatment History Monitoring**

Incident	Location/ Substrate	Type of Oil	Type of Bioremediation	Products	Days Monitored	Endpoints Measured	Application Effective?
Exxon Valdez	Prince William Sound, Alaska shorelines	Prudhoe Bay crude	fertilizer	Inipol Customblen	1989: 99 days 1990: 55 days	microbial counts respirometry GC/MS water quality bioassays (acute) chlorophyll oil residue weight	yes, partially
Prall's Island	Arthur Kill, New Jersey gravel beach	fuel oil	fertilizer	Customblen	92 days	water quality TPH microbial counts GC/MS	inconclusive
Apex Barges	Galveston Bay, Texas marsh	partially refined (catalytic feed stock)	microbial	Alpha BioSea with Miracle-Gro	9 days	bioassays (acute) percent oil in mousse TPH GC/MS Fatty acids	no
Seal Beach	Southern California marsh	crude	microbial	INOC 8162 Miracle-Gro	35 days	respirometry stable isotope ratio phenanthrene mineralization microbial counts	no
Mega Borg	Gulf of Mexico open water	Angolan crude	microbial	Alpha BioSea	7 hours	bioassays (acute) percent oil in mousse	inconclusive

(DuPont, 1991; Goodbred, 1991; Mearns, 1991; Nadeau, 1991; Parris and Albrecht, 1990; Prince *et al.*, 1990; Pritchard, 1991; Pritchard *et al.*, 1991; Texas General Land Office, 1990; U.S. EPA, 1991) cases. It may be concluded that bioremediation has an important role to play as a long-term treatment for many problem environments in oil spill cleanup, such as in sensitive environments, and those with subsurface oil. As an additional countermeasure, it is a useful, versatile, and still-developing tool.

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