Pritchard, P.H. 1993. Effectiveness and Regulatory Issues in Oil Spill Bioremediation: Experiences with the Exxon Valdez Oil Spill in Alaska. In: Biotreatment of Industrial and Hazardous Waste. Morris A. Levin and Michael A. Gealt, Editors, McGraw-Hill, New York, NY. Pp. 269-307.

EPA/600/A-94/205

Chapter

12

Effectiveness and Regulatory Issues in Oil Spill Bioremediation: Experiences with the Exxon Valdez Oil Spill in Alaska

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Abstract

The use of bioremediation as a supplemental cleanup technology in the *Exxon Valdez* oil spill, in Prince William Sound, Alaska, has proven to be a good example of the problems and successes associated with the practical application of this technology. Field studies conducted by scientists from the U.S. Environmental Protection Agency have demonstrated that oil degradation by indigenous microflora on the beaches of Prince William Sound could be significantly accelerated by adding fertilizer directly to the surfaces of oil-contaminated beaches. Our results from the application of an oleophilic fertilizer are presented as exemplary field and laboratory information. The fertilizer enhanced biodegradation of the oil, as measured by changes in oil composition and bulk oil weight per unit of beach material, by approximately twofold relative to untreated controls.

These studies supported bioremediation as a useful cleanup alternative that was subsequently used by Exxon on a large scale. They have

also generated a number of insightful lessons that have significant relevance to future oil bioremediation efforts. This chapter discusses these lessons and examines complications and difficulties in assessing the effectiveness of bioremediation in the field.

Further field studies at a site involving an oil-contaminated beach that was less energetic and higher in nonpetroleum organic matter and using slow-release fertilizer granules applied at different concentrations, contrastingly showed little effect of fertilizer application. Precautions regarding extrapolation either from the laboratory to the field or from field site to field site, are discussed.

As with many types of bioremediation, protocols are needed to generate consistent and relevant data sets for commercial processes that will allow appropriate decisions to be made relative to the use of the process or product in a field cleanup operation. The conceptual basis for these protocols is a complicated matter and its development is significantly influenced by field experiences such as the *Exxon Valdez* oil spill. Discussion of these concepts provides an informative picture of the problems and assumptions faced in making decisions about when and how to apply a bioremediation technology.

The use of bioremediation for the cleanup of soils, sediments, and aquifer materials contaminated with oil and petroleum hydrocarbons has been extensively recognized (Lee and Levy, Bartha and Pramer). Success has been possible because of the relative biodegradability of oil and the knowledge that hydrocarbon degraders can be enriched in many, if not most, types of environments (Levy, Atlas). In addition, bioremediation is gaining acceptance as a viable technology; if used prudently, it can provide efficient, inexpensive, and environmentally safe cleanup of waste chemicals. Thus, the suggestion to use bioremediation as a supplemental cleanup tool in the *Exxon Valdez* oil spill in Prince William Sound, Alaska, was readily accepted as use of a new technology ready for field demonstration (Pritchard and Costa). The implementation of field studies to establish that oil degradation by indigenous microflora on the beaches of Prince William Sound could be significantly accelerated by fertilizer application (Pritchard et al., 1991), and the eventual large-scale application of fertilizer by Exxon as part of their overall cleanup program, provided a number of useful lessons and experiences that, if considered in the proper light, could have considerable influence on future oil bioremediation efforts.

The emphasis of this chapter will be on some of the difficulties and problems associated with the fertilizer application and its effect on oil degradation. I will concentrate primarily on the separate application of an oleophilic fertilizer which occurred at a site called Snug Harbor on Knight Island in Prince William Sound, and on the application of slow-release fertilizer granules which occurred on Disk Island in Prince William Sound. These applications provide contrasting results that are instructive for both their success and failure. (Note that additional fertilizer applications were conducted at these and other sites and summaries of these results are available; Pritchard et al., 1991).

Closely linked to these field applications of fertilizers is the question of which commercial products should be used and how the best ones should be screened out. This includes not only fertilizers but also microorganisms and other oil-biodegradation-stimulating agents and concepts. In Alaska, many of these commercial products could not be considered because of the very short time available for field demonstrations, but also because the data available for each product were so variable and/or insufficient that reasonable selections could not be made in a timely fashion. Subsequent to the *Exxon Valdez* oil spill, however, the Office of Research and Development of the U.S. Environmental Protection Agency (EPA) embarked on the development of effectiveness and environmental safety testing protocols that could be used to establish a consistent and relevant database upon which decisions for the use of particular commercial products might be based in the future. Some of these protocols have been developed and are currently being validated with laboratory studies. The development of a conceptual basis for these protocols has proven to be a useful exercise that depends heavily on the lessons learned in Alaska and other oil spills. Describing, in part, that conceptual development here provides an additional dimension to the regulatory complications that come into play in the application of bioremediation to oil spill cleanup. It is my hope that this information will stimulate others to carefully consider the process by which bioremediation success is measured in the field. It is this success issue, along with environmental safety aspects, that will be the key to good regulatory decision making.

Background

In any bioremediation effort, success will invariably involve a scientifically valid demonstration of process effectiveness and environmental safety. Effectiveness, in the case of oil bioremediation, means establishing that (1) removal or disappearance of the oil is attributable to biodegradation and not other nonbiological processes and (2) enhanced biodegradation rates of oil are sufficiently better than natural rates to justify expenditure of effort to implement the bioremediation process on a large scale. Although environmental safety issues will not be addressed here, they too require considerable effort to verify the absence of any adverse ecological effects associated with the fertilizer application.

In the *Exxon Valdez* oil spill, both aspects were crucial to the eventual acceptance of bioremediation by the public and state and federal regulatory agencies. Quelling skepticism, given the "subtleties" of a biotechnological approach, indeed was and will continue to be a chal-

lenge in almost any bioremediation situation, whether it deals with oil or other types of chemicals. Effectiveness and environmental safety issues for bioremediation in Prince William Sound, where treatment was centered on oil-contaminated gravel and cobblestone beaches, will, of course, be considerably different than for oil-contaminated sandy beaches, marshes, and wetlands. However, the lesson learned in terms of demonstrating a viable cleanup technology will have no bounds.

Reflections on the initial assumptions by EPA scientists and their colleagues as planning of the project commenced give rise to important and useful insights. Several assumptions, discussed in the context of what actually occurred in the field, provide instructive lessons that could impact the responses to bioremediation at other spill sites. While confidence provided by almost twenty years of accumulated research data on oil biodegradation laid the groundwork for these assumptions, we would have been naive not to expect some surprises!

Enrichments of oil-degrading microbial communities

Clearly, it was reasonable to expect, even in the cold water temperatures of Alaska, a significant enrichment of oil-degrading microorganisms in the beach material following exposure to the oil. Research by Atlas and his colleagues supported this idea (Atlas, 1981). As it turned out, by early June 1989 (approximately 2 months after the spill), concentrations of oil degraders averaged around 10^6 per gram of oiled beach material, which represented as much as a 10,000-fold increase in the number of degraders relative to beaches that had not been contaminated with the oil. Studies by Lindstrom et al. (1991) have shown similar trends, and an example of their results is shown in Table 12.1. Enrichments of this magnitude suggested that oil was being degraded (previous studies have demonstrated that Prudhoe Bay crude oil is quite biodegradable), that some nitrogen and phosphorus were available to support growth of the hydrocarbon degraders, and that the cold temperatures (10–16°C) were probably not overly restrictive to the indigenous microflora. The information not only implied the possibility of nitrogen-limited biodegradation (i.e., a great excess of degradable organic carbon from the oil in the face of a finite supply of nitrogen in the water), but also opened the possibility of accelerating oil biodegradation by overcoming this limitation alone.

In this case, we believed it was unnecessary to experimentally reverify, through laboratory studies, the stimulatory effect of nitrogen on oil biodegradation, even in oil-contaminated beach samples from Prince William Sound, since previous experiences and numerous published reports in the literature supported this as a sensible approach. Instead,

TABLE 12.1 Median (n = 5 to 9 Samples) Hydrocarbon-Degrader MPN Microbial Counts per Gram (Dry Weight) of Sediment for Treated (T) and Reference (R) Plots*

Beach site	Day sampled	Median MPN cells (10^4) per g of surface sediment T	R	Different?†	Median MPN cells (10^4) per g of subsurface sediment T	R	Different?
KN-135B	0	2.62	4.24	No	1.66	1.63	No
	2	4.79	1.58	No	1.02	0.47	No
	4	15.50	4.20	No	10.30	1.00	Yes
	8	1.56	15.60	No	10.10	2.27	No
	15	15.60	9.75	No	16.20	2.34	No
	52	13.70	23.40	No	75.40	36.00	No
	56	139.00	17.90	Yes	582.00	9.78	Yes
	70	149.00	25.20	Yes	126.00	13.00	Yes
	78	185.00	122.00	No	170.00	117.00	No
KN-211B	0	0.96	4.63	No	4.60	1.63	No
	2	77.00	127.00	No	193.00	2.23	Yes
	4	9.55	48.00	No	81.93	80.35	No
	16	45.40	44.44	No	46.22	97.49	No
	31	23.94	98.78	No	99.80	24.95	No
	45	30.83	53.23	No	33.18	25.32	No
	102	18.10	8.51	No	3.72	0.96	No
	112	3.19	11.70	No	8.51	1.28	Yes
KN-132B	0	24.90	23.00	No			
	2	155.00	21.70	No			
	4	77.70	16.00	No			
	8	160.00	37.10	No			
	16	97.30	15.70	Yes			
	29	28.00	16.00	No			
	43	135.00	0.59	Yes			
	60	84.10	1.78	Yes			
	95	117.00	53.20	Yes			

*All values obtained for each day were subjected to a Mann-Whitney two-sample U test to determine whether the sampled populations were different at the 95% confidence level. KN-135B was initially treated after day 0 and was refertilized after day 52. KN211B was initially treated after day 0 and refertilized on day 42. KN132B was fertilized after day 0 and again on day 40.

†Statistically significant differences are reported for surface and subsurface sediments.

we reasoned that, in light of the magnitude of the problem at the time, it was better to go directly to the field and conduct a practical demonstration of the same principle. Thus, in the very early stages of the oil spill cleanup program in Alaska, the relatively simple measurement of the number of oil degraders, along with the relevant literature information, had provided a reasonable "first-line" indicator of oil bioremediation feasibility.

Mineralization test as an indicator of oil degradation activity

Depending on the environmental situation involved in the oil spill, other first-line indicators of bioremediation potential might be desirable. There is always a tendency, where the local public is involved, to generate "site-specific" information, at the very least to generate a better comfort factor. A discussion of these indicators here, therefore, is worthwhile, even though they were not used initially in Alaska. Circumstances are likely to occur in other spills in which information on the enrichment of oil degraders in the contaminated areas alone is not sufficient for initial decision-making purposes.

Mineralization studies involving measurements of total CO_2 production can provide excellent first-line information. The approach provides, quick, relatively unequivocal time course data suitable for testing different treatment options (e.g., effects of adding nitrogen). If natural oil degradation is occurring in contaminated beach material, then considerable amounts of CO_2 should be produced from oil mineralization relative to a control containing uncontaminated beach material. Several laboratory systems can be used for this type of measurement. Biometer flask systems (Bartha and Pramer, 1965), which are designed to trap CO_2 in side-arms containing an alkaline solution, can be adapted to measure these mineralization rates of oil on contaminated beach material (Mueller et al., 1992). Commercially available respirometric systems, such as the Micro-Oxymax™ (Columbus Instruments, Columbus, Ohio) can also be used. The Micro-Oxymax™ system, which can be adapted to standard laboratory shake flasks, also measures oxygen consumption. The procedure entails placing oil-contaminated beach material and its associated microbial community (mixed sand and gravel in the case of Prince William Sound) directly in the flasks and flushing fresh seawater in and out as a simulation of the tidal exchange (Mueller et al., 1992). An example of the data generated from such a mineralization test is shown in Fig. 12.1. This experiment was performed with oil-contaminated Prince William Sound beach material, taken considerably after our bioremediation field demonstration had begun. The respirometric flask method is indicative of the short-term tests that can be initially conducted as a data-gathering exercise for a particular oil spill. Note the enhancing effect of adding nitrogen fertilizer (Fig. 12.1). By comparing rates of CO_2 production, an estimate of the extent of enhancement of oil biodegradation can be obtained. The system can be easily adapted to test beach material from the other oil spill sites.

Since any other type of organic matter in the beach material can also produce CO_2, care must be taken to assure that one is measuring oil

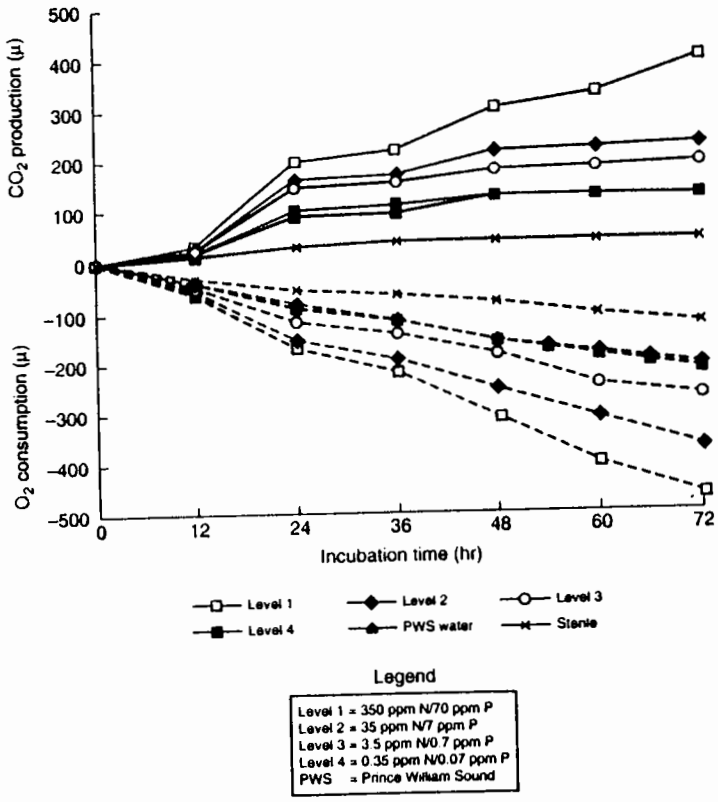


Figure 12.1 Fertilizer specific activity (O_2 consumption, CO_2 production) for six treatments over time.

mineralization. In addition to running control flasks with uncontaminated beach material as indicated above (with and without added nitrogenous nutrients) and ensuring that mineralization in the presence of the oil is considerably above background, one can also add a radiolabeled hydrocarbon. If oil degradation is active, the production of radiolabeled CO_2 should be extensive and immediate. We have found that phenanthrene works well because it was rapidly and completely absorbed to the oil (Mueller et al., 1992; Pritchard et al., 1991). The tidal cycle washed out any phenanthrene remaining in the aqueous phase, and subsequent CO_2 production was then mainly from the bacterial communities associated with oil surfaces.

Oil spill bioremediation as a finishing step

We also made the very important initial assumption that bioremediation would be most effective as a finishing step in the cleanup program. As it turned out, the physical washing procedure employed by Exxon removed the bulk of the oil, but it left the beach material still quite contaminated and aesthetically unpleasing, and the oil ecologically available. As there were very few follow-up alternatives, bioremediation then became intriguing as a finishing step. Without the initial removal of this bulk oil, bioremediation may not have been tenable in the context of Alaska. That is, even if oil biodegradation was quite active, it is largely a surface-oriented process and it would take extended time (there was a relatively small window in the summer months in Alaska when water temperatures are amenable to oil biodegradation). For quicker results, the oil must first be distributed throughout the beach material to increase the surface-to-volume ratio. And since tilling was unreasonable on most of the cobblestone beaches in Alaska, the more bulk oil removed by a physical cleanup process and the more the residual oil dispersed into the beach material, as was accomplished by the physical washing procedure, the more effective bioremediation would likely be. Similar considerations would be required for other types of beach material.

In highly porous beaches, as found in Prince William Sound, oil diffuses to some extent into the gravel, thus increasing the contaminated surface area. The physical washing process may enhance this aspect. On a sand or mud shoreline, however, where porosity is much lower, most of the oil will predictably be concentrated at the surface. Initially, the contaminated beach material may very well be physically removed. But the remaining contaminated beach material can potentially be treated effectively by bioremediation, again as a finishing step. In this case, tilling may be used as a mechanism to further disperse the remaining oil into the beach material, increasing the surface-to-volume ratio, and improving the success of bioremediation. Tilling also aerates beach material and helps disseminate added fertilizers, thus preventing the availability of oxygen or inorganic nutrients from becoming a major limiting factor to bioremediation. In Alaska, because of the highly porous nature of the beaches, the high oxygen concentrations in the cold seawater, and the flushing effect of 5-m tides, oxygen limitation was never a consideration.

Choice of Fertilizer Formulations

We assumed for Alaskan beaches that nitrogen (and phosphorus) had to be applied in a manner which would passively expose the oil-degrading microbial communities to the elevated nutrient concentrations

TABLE 12.2 Description of Fertilizers Tested

	Commercial Name		
	Woodace	Customblen	Inipol EAP 22
Manufacturer	Vigoro Industries	Sierra Chemical Co.	Elf Aquataine
Form	Briquette	Granule	Liquid
Size	5 × 5 × 5 cm	2- to 3-mm diameter	—
N source	Isobutyraldehyde-diurea	Ammonium nitrate	Urea
N:P:K	14:3:3	28:8:0	7.3:2.8:0
Specific gravity	1.8	1.8	0.996 g/mL
Viscosity	—	—	250 cSt
Application rate on 12-m × 35-m plots	986 g/m^2	100 g/m^2	284 g/m^2
Method of application	Net bags (11.8 kg. ea.)	Fertilizer spreader	Backpack sprayer
Test areas	Snug Harbor	Snug Harbor Passage Cove	Snug Harbor Passage Cove

over an extended period. Given the large tidal cycle and significant wave action, fertilizer materials placed on the beach surface would likely wash away in a few days. To overcome this problem, two types of slow-release fertilizers were initially considered; solid pelletized formulations and liquid oleophilic formulations. Characteristics of each fertilizer considered are summarized in Table 12.2.

Summarizing some of the criteria used to make the selections is instructive, as this could help in making fertilizer selections in the future. The three main criteria were (1) ease of application and potential to retain position on the beaches, (2) nutrient release characteristics, and (3) physical durability over time. As it turned out, fertilizer granules seemed to best fit the criteria (Pritchard et al., 1991). They were easy to apply over a large surface area and were found to stick tightly to the oiled beach material and worked their way down under cobble where they were difficult to dislodge. Nutrient release characteristics, which were determined in simple laboratory test systems (Venosa et al., 1990; Glaser et al., 1991), showed that much of the nitrogen (ammonia and nitrate) and phosphorus (phosphate) release from the granules occurred in the first 24 to 72 h (Fig. 12.2). However, sufficient quantities continued to be released steadily for considerable periods thereafter. Thus, as the tides wash in and out of the beach, nutrients should be distributed to the microbial communities associated with the oil for a period of 2 to 3 weeks or longer. Although the physical condi-

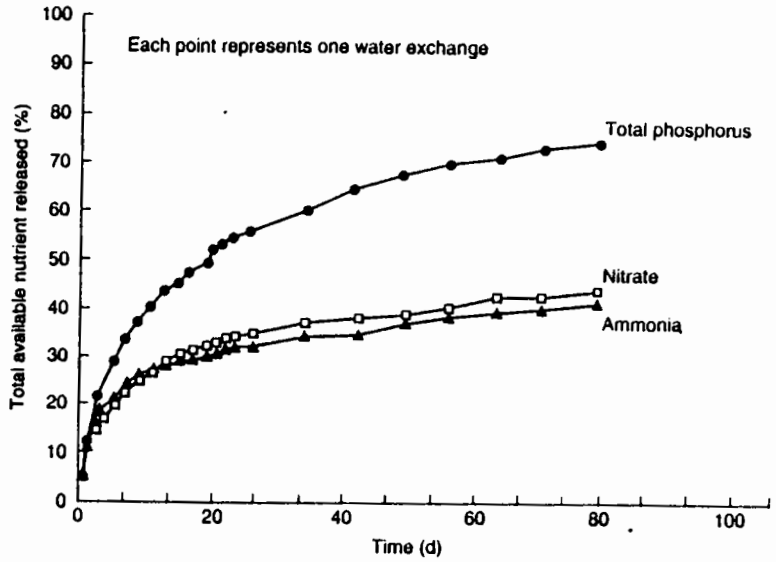


Figure 12.2 Cumulative release of ammonia and nitrate from SIERRA CHEMICAL granules in static flask equipments.

tion of the granules slowly deteriorated on contact with seawater, we observed granules on the beaches with fertilizer inside 2 to 3 weeks after application. The fertilizer granules were ultimately used by Exxon in combination with the liquid oleophilic fertilizer for all of the large-scale applications in Prince William Sound.

Fertilizer briquettes were also found to be satisfactory based on the above criteria (Venosa et al., 1990). These are approximately the size of charcoal briquettes and contain organic sources of nitrogen and phosphorus that slowly hydrolyze to release urea and phosphate over time. Nutrient release characteristics of the briquettes were very similar to those of the granules. Although the briquettes maintained their physical integrity much better, if broadcast over the beach surface, they were easily moved around by the tides and waves, resulting in very heterogeneous distributions. Consequently they had to be packaged in seine net bags and secured to the beach with metal stakes. Although this was effective, it presented significant logistical problems at the time of their consideration for use on a large scale. The briquettes were, however, used as part of our field demonstration in bioremediation, and appeared effective in enhancing oil biodegradation (Pritchard et al., 1991).

Rather than broadcast fertilizer granules and briquettes onto the beach surface, they could also be buried in the beach material, say in

trenches running parallel to the water line in the contaminated intertidal zone. Depending on the porosity of the beaches, tides and interstitial water movement could effectively distribute the released nutrients to the bacteria. Because of the physical integrity of the briquettes, they would be most suitable for this type of application. A burial approach was initially considered in Alaska but never really tested, again because of perceived logistical restrictions (later to become unfounded). However, fertilizer granules were applied by Exxon and the State of New York in this manner to a sandy beach on Prall's Island (located in an estuary southwest of New York City) that was contaminated with diesel fuel. Initial reports (Madden, 1991) suggested that the application was successful in enhancing diesel oil biodegradation. Distribution of nutrients will, of course, depend on the hydrodynamics of interstitial water in the beach, and, in many cases, specific information will be lacking. Rather than proceeding based on these limited successes, one could quite easily perform a pilot study, of several days' duration, to actually measure movements of nutrients in interstitial water (Madden, 1991).

Pragmatically, the best criteria for determining how much slow-release fertilizer to place on a given beach were to apply as much as possible without exceeding toxic concentrations of ammonia and/or nitrate. These nutrients are toxic (96-h LC_{50}) to marine invertebrates (good sentinel bioassay for most sensitive species) at concentrations in the water of around 10–15 ppm (Pritchard et al., 1991). Our experience has been that one will face adverse environmental effects thresholds long before the demand for nitrogen by the oil-degrading microbial communities is saturated. Keep in mind that the toxic effect threshold should take into account the initial burst release of nutrients associated with these types of fertilizer formulations. Alternatively, determining fertilizer application rates based on the quantity of oil present in the environment is made difficult because of the tremendous heterogeneity in oil distribution and concentration frequently encountered in the field.

Oleophilic fertilizer

The concept of oleophilic fertilizers is based on the use of organic sources of nitrogen and phosphorus in a liquid carrier that is miscible with oil. In theory, when the liquid carrier is applied, the nutrients essentially dissolve into the oil and thereby keep them in contact, for sustained periods, with the bacteria growing on the oil's surface. Several types of oleophilic fertilizers have been successively tested in both laboratory and small-scale field experiments. Most of these were designed and tested with the idea of treating oil on the surface of water rather than oil on beach material. Pioneering studies by Atlas and Bartha

(Atlas and Bartha, 1973), demonstrated that the addition of paraffinized urea and octylphosphate to Prudhoe Bay crude oil on the water surface significantly enhanced the biodegradation of the oil. Similar success has been reported for a commercial product, Victawet 12, or 2-ethylhexyl-dipolyethylene oxide phosphate (Bergstein and Vestal, 1978), for several natural sources of lipophilic nitrogen and phosphorus, such as soybean lecithin and ethyl allophanate (Olivieri et al., 1978), and for $MgNH_4PO_4$ incorporated into a paraffin support base (Olivieri et al., 1976). Uncertainties related to the specific mechanisms of enhanced oil degradation, the factors affecting physical release rates of the nutrients in situ, and the effect of adding large quantities of organic carbon to the oil, have perhaps limited the application of these products. In addition, several reports comparing enhanced oil degradation by oleophilic and regular fertilizer showed little difference (Lee and Levy, Halmo). Therefore, the advantage of using oleophilic fertilizers has not been clearly established. Nonetheless, the concept of oleophilic fertilizers was intriguing to us given that it was the prevailing method for placing nutrients directly in contact with the microbial communities, and thus further field-testing seemed justified.

We selected the oleophilic fertilizer Inipol EAP 22, produced by Elf Aquataine Company in France, since it was the only commercially available source with large production capability at hand. This unique product is a stable microemulsion consisting of a core of urea (the nitrogen source) surrounded by an oleic acid carrier. Laureth phosphate (a surfactant and the source of phosphorus) acts as an emulsion stabilizer and butoxy ethanol (methyl cellusolve) reduces viscosity. This formulation has shown good promise in tests conducted in the laboratory and in large outdoor tanks using different types of oil-contaminated beach material and environmental locations (Lee and Levy, 1987; Sveum and Ladousse, 1989; Tramier and Sirvins, 1983). Interesting results were obtained from a test conducted on an oil-contaminated beach in Spitsbergen, Norway (Sveum and Ladousse, 1987); Inipol appeared to increase oil biodegradation rates when applied to coarse-grained gravelly beach material but not when applied to fine-grained sandy beach material. Based on these studies, Inipol appeared to have significant potential for bioremediation. When Inipol was applied to oil-contaminated mixed sand and gravel from Prince William Sound in laboratory studies designed to provide intermittent submersion with seawater, approximately 60 percent of the urea (measured as total Kjeldahl nitrogen, TKN) was released within the first few minutes after application (Table 12.3). Measurements of total phosphorus showed a similar percentage. A 6-h wait before the first submersion produced the same results as a 5-min wait, suggesting that the Inipol "sets" quite quickly. However, following this initial burst of TKN and

TABLE 12.3 Release of Ammonia, Total Kjeldahl Nitrogen (TKN), and Total Phosphorus (TP) from Inipol EAP 22 during Intermittent Submersion Experiment

Minutes from start of experiment	5-min contact time*	6-h contact time*
Ammonia released (mg N/L)†		
5	1.1	0.5
15	1.1	0.4
30	1.4	0.5
60	1.3	0.7
120	1.4	0.7
510‡	0.2	
540	0.1	
600	0.0	
TP released (mg P/L)		
5	1.3	1.4
15	1.2	1.2
30	1.0	1.1
60	1.5	1.3
120	1.0	1.0
510‡	1.1	
540	0.9	
600	0.9	
TKN released (mg N/L)†		
5	24.6	29.8
15	26.1	34.8
30	27.2	35.5
60	32.5	34.3
120	29.4	32.3
510‡	4.6	
540	4.6	
600	4.3	

*Time between fertilizer application and initial submersion.

†Initial concentration of nitrogen = 57 mg/L.

‡Water drained; beach material remained unsubmerged for 6 h; seawater replaced.

phosphorus, there was essentially no release of these materials. Presumably, the residual nitrogen and phosphorus, although tightly held to the oiled beach material, was in fact available to the bacteria degrading the oil. Experimentally demonstrating this availability was difficult, as will be discussed below.

Inipol application in Prince William Sound was initially conducted with a backpack sprayer to give a thin coating over the oiled beach material (Pritchard et al., 1991). Appropriate coatings could be controlled because the oil appeared wet and became a deeper black in color following coverage by the Inipol. This visual effect disappeared several hours after application. In the large-scale use of Inipol by Exxon, an application rate of approximately 0.3 L/m^2 was used, based, in large part, on obtaining the surface coverage used in our field demonstration project.

Visual changes. Test beaches at Snug Harbor, where Inipol was applied as part of a field demonstration of its effectiveness, produced some surprising visual results (Pritchard and Costa, 1991). These beaches were moderately contaminated with oil and had not been subjected to the physical washing process at the time of our test. They were selected as representative of those that received the physical washing. Visually, the cobble areas had a thin coating of sticky oil covering the rock surfaces and mixed sand and gravel under the cobble. Oil did not penetrate more than a few centimeters below the gravel surface. In some areas, small patches of thick oil and "mousse" (oil/water/air mixture in colloid form) could be found.

Approximately 10 to 14 days following oleophilic fertilizer application, reductions in the amount of oil on rock surfaces were visually apparent. The change was particularly evident from observations in aircraft where the contrast with oiled areas surrounding the plot was dramatic, etching a "clean" rectangle (12 × 28 m) on the beach surface. The contrast was also impressive at ground level; there was a precise demarcation between fertilizer-treated and untreated areas. At this time, the untreated control plots appeared unaltered visually.

Close examination showed that much of the oil on the surface of the cobble was gone, yet considerable amounts of the oil remained under the cobble and in the mixed sand and gravel below. Remaining oil was not dry and dull as was the oil on the untreated control beach, but appeared softened and wetter. It was also very sticky to the touch, with no tendency to come off the rocks. At the time of these observations, no oil slicks or oily materials were observed leaving the beach during tidal flushing.

We believe that visual disappearance of oil on the cobble surface 2 to 3 weeks following Inipol application was largely due to biodegradation and not a chemical washing phenomena. Chemical data to support this belief are presented below. In addition, we tried to force the chemical washing effects by adding large concentrations of Inipol repeatedly on several miniplots in Snug Harbor, and it did not affect oil removal; a period of at least 2 to 3 weeks was required to see any "cleaning" effect regardless of the amount of Inipol applied. The application of aqueous fertilizer solutions (tested at a different beach), which contained only inorganic chemicals and no organic-surfactant-like materials, also produced the "cleaning" effect in about the same time period, further supporting the role of biodegradation (Pritchard et al., 1991). Finally, experiments performed by Exxon researchers (R. Prince, S. Hinton, and J. Bragg, personal communications) have shown that, in specially designed tests to measure the effectiveness of a variety of commercially available chemical rock washers, Inipol was ineffective. Also, they have observed in microcosm studies that Inipol seemed to become more tightly associated with the beach material; that is, the oil had much more of a tendency to move to the glass walls of the microcosms in the absence of Inipol.

Six to eight weeks after fertilizer application, the contrast between the treated and untreated areas on the cobble beach had lessened. Reoiling of the Inipol-treated beach from oiled subsurface material and/or the concurrent slow removal of oil on the surface of the beach material surrounding the treated areas was probably responsible for this decrease in contrast. Toward the end of the summer season, the area used for the bioremediation studies became steadily cleaner, including the control plots. Several storms and more frequent rainfall, as well as natural biodegradation, undoubtedly contributed to these changes.

Overall, rapid oil disappearance brought on by the application of the oleophilic fertilizer made these beaches more compatible with local wildlife (less tendency for fur and feathers to become oiled). These dramatic changes occurred in a shorter period of time than the limited changes noticed in untreated plots, and possibly helped accelerate biological recovery of the intertidal area.

Measures of Effectiveness

Obtaining definitive information on the role of biodegradation in the removal of oil residues from beach material, or from any complex environmental matrix for that matter, is a difficult task. In general, for oil spill bioremediation, one has to produce *both* qualitative information on changes in oil composition that are indicative of biological processes *and* quantitative information on decay rates of oil, or some of its hydrocarbons, that are also indicative of biological processes. Qualitative information establishes the extent to which biodegradation has occurred; however, with a complex chemical mixture like Prudhoe Bay crude oil, the removal of more than just a few short-chain hydrocarbons (representing only a small percentage of the oil), and removal of more than just the aliphatic fraction of the oil (i.e., leaving behind aromatic, heterocyclic, and branched hydrocarbons, polar chemicals, etc.), is desirable.

Simultaneously the quantitative information establishes that the enhancement of oil biodegradation by the fertilizer treatments was sufficient to merit full-scale operation; generally a two- to threefold enhancement over the untreated controls will probably be acceptable to many decision makers and regulatory groups, but this is not based on a comprehensive database. Both types of information, however, were difficult to obtain in the field because of many different problems encountered.

Any bioremediation testing program that is based on analytical techniques involving the disappearance of oil residues or the disappearance

of hydrocarbons resolvable by gas chromatograph is open to scientific criticism because several environmental fate processes (including photosynthesis, physical dissolution, chemical washing, volatility, etc.) can affect or contribute to this disappearance phenomenon. To confront these potential criticisms, we chose an approach that integrated several analytical procedures with several key assumptions. First, we assumed that the disappearance of several target hydrocarbon groups could be used as definitive indicators of biodegradation. We assumed further that strong indicators of biodegradation would be associated with substantial changes in the composition of several fractions in the oil, particularly selected aromatic hydrocarbons. We would thus attribute these compositional changes to biodegradation.

Second, we assumed that if a correlation between changes in hydrocarbon composition and changes in residue weight of the oil could be established, disappearance rates of the residue weights could be used as the primary quantitative measure of fertilizer effect (i.e., significant differences between treated and control plots). The rate of information could then be used to estimate cleanup effectiveness over extended time periods.

However, some discussion as to why these parameters were selected is in order because the criteria for what constitutes biodegradation of oil are complicated and controversial. Many studies have considered measurements of reductions in aliphatic hydrocarbon concentrations as generally indicative of biodegradation, but their value is often questioned because these hydrocarbons are (1) frequently the most readily degradable fraction, (2) the least toxic, and (3) often only a small percentage (by weight) of the oil. Measuring compositional changes in the aromatic fraction adds a further dimension, as these hydrocarbons are less readily degradable and potentially more chronically toxic. However, most of the common procedures for measuring the aromatic hydrocarbons are based on mass spectral analysis which concentrates on only 10 to 20 selected compounds, representing only a very small fraction of the total aromatics. Whether these selected aromatic hydrocarbons are good surrogates for the degradation of the rest of the aromatic compounds has not been established. However, if one concentrates only on the aromatic hydrocarbons and shows that they degrade, one has the advantage of being able to assume that aliphatic hydrocarbons will almost certainly be extensively degraded as well.

Regardless of compositional changes, it seems reasonable to require that bioremediation, as a worthy cleanup tool, should effect the removal of bulk material; changes in composition without much change in oil residue removal seem to present only half the picture. Moreover, oil biodegradation under optimized conditions in the laboratory will result in as much as a 40 to 60 percent reduction in the total weight of oil

(Atlas and Bartha, 1973) and therefore it does not seem unreasonable that some reduction in oil residue can be expected, even under field conditions. The ultimate measure of biodegradation would be to fractionate the oil into aliphatic, aromatic, heterocyclic, polar, and asphaltene fractions and determine weight loss of each of these fractions. Most of these fractions can be analyzed by gas chromatography to determine qualitative changes in composition. This analytical procedure has been used by Westlake and his colleagues in several studies (Jobson et al., 1972).

Changes in the normal-alkane-to-branched-alkane ratios

To provide perspective on measuring effectiveness in bioremediation at the level of a field demonstration, the results from Alaska with the oleophilic fertilizer are instructive. Chemical analysis of the oiled beach material, exposed and unexposed to the oleophilic fertilizer, was accomplished by collecting beach material according to a block design (21 samples taken at each sampling time, 7 each in contiguous blocks along a line in the high-, mid-, and low-tide zones of the beach) and then extracting samples (with methylene chloride) from the cobble surface and from the mixed sand and gravel under the cobble. The weight of oil recovered (measured in milligrams per gram of beach material) was determined gravimetrically and oil composition was determined by injecting extracts into a gas chromatograph following standard analytical procedures (Pritchard et al., 1991).

Changes in composition were determined first by examining the resolvable alkanes. Historically, this has been done by calculating the weight ratio of a hydrocarbon that is known to readily biodegrade (generally the C17 and C18 normal alkanes) to one that is slower to biodegrade (generally the branched alkanes pristane and phytane), which chromatograph very close to the n-C17 and n-C18 alkanes) (Atlas). We generally focused on the n-C18/phytane ratio because pristane is sometimes found naturally in seawater. The ratio concept is based on the idea that most nonbiological fate processes (physical weathering, volatilization, leaching, etc.) will not produce differential losses of aliphatic and branched hydrocarbons that have similar gas chromatographic, and correspondingly, chemical, behavior. Support for this concept can be found in the biogeochemical studies on oil (Kennicutt, 1988). However, since the branched alkanes do in fact biodegrade (Prinik et al., 1977; Mueller et al., 1992), they need only to degrade more slowly than the straight-chain alkane to take advantage of the ratio method. Clearly, in this case, the measure of biodegradation will be conservative.

Focusing on the effects of the oleophilic fertilizer Inipol EAP™ 22, the results in Fig. 12.3*a* show that, following an initial lag, extensive decay in the n-C18/phytane ratio occurred through time for cobble surface samples. A decay also occurred on the untreated control beach but at about half the rate on the fertilizer-treated beach (Fig. 12.3*b*). Despite the large variability around the median values, slopes of the decay curves (following the June 17 sampling and not including the September 9 sampling) were statistically different from zero and from each other at the 95% confidence interval. Based on the assumptions described above about the meaning of the ratio changes, biodegradation was occurring on both beaches and was enhanced by the application of the fertilizer. Note that oil had already undergone biodegradation prior to fertilizer application as the ratio for undegraded weathered Prudhoe Bay crude was around 2.0. Also, large decreases in the ratios were invariably linked to considerable reduction, if not complete removal, in the concentrations of the resolvable (by gas chromatography) alkanes, n-C17 to n-C30.

The large variability in the ratios shown in Fig. 12.3 (that is, many samples showed evidence of biodegradation while others showed very little) was a function of the highly heterogeneous distribution of oil on the beach. Possibly the same amount of biodegradation was occurring in each sample, but since biodegradation takes place on the oil's surface, a grab-sampling procedure (which was almost unavoidable in this case) necessarily encompasses sufficient quantities of undegraded oil from below that surface to dilute the measure of biodegradation.

Much less change in the n-C18/phytane ratio, if any, occurred in the mixed sand and gravel under the cobble for the oleophilic-fertilizer-treated beach and the untreated control (Fig. 12.4*a* and 12.4*b*). As striking, however, was the unexpected difference in the initial ratio between the cobble surface samples and the mixed sand and gravel samples (t = 0 sampling, June 8, 1989). In both cases, substantial biodegradation of the oil had occurred prior to fertilizer application but the degradation was much more pronounced in the mixed sand and gravel samples. Why the ratio was so much lower in the mixed sand and gravel was not clear. With less total oil concentration overall in these samples initially, biodegradation was possibly more apparent because of less dilution from undegraded oil during sampling.

Biodegradation of phytane

Following the logic set out above, the absence of a change in the n-C18/phytane ratios through time for the mixed sand and gravel samples suggested that oil biodegradation was not occurring despite its degraded state prior to fertilizer application. The initial low ratio may

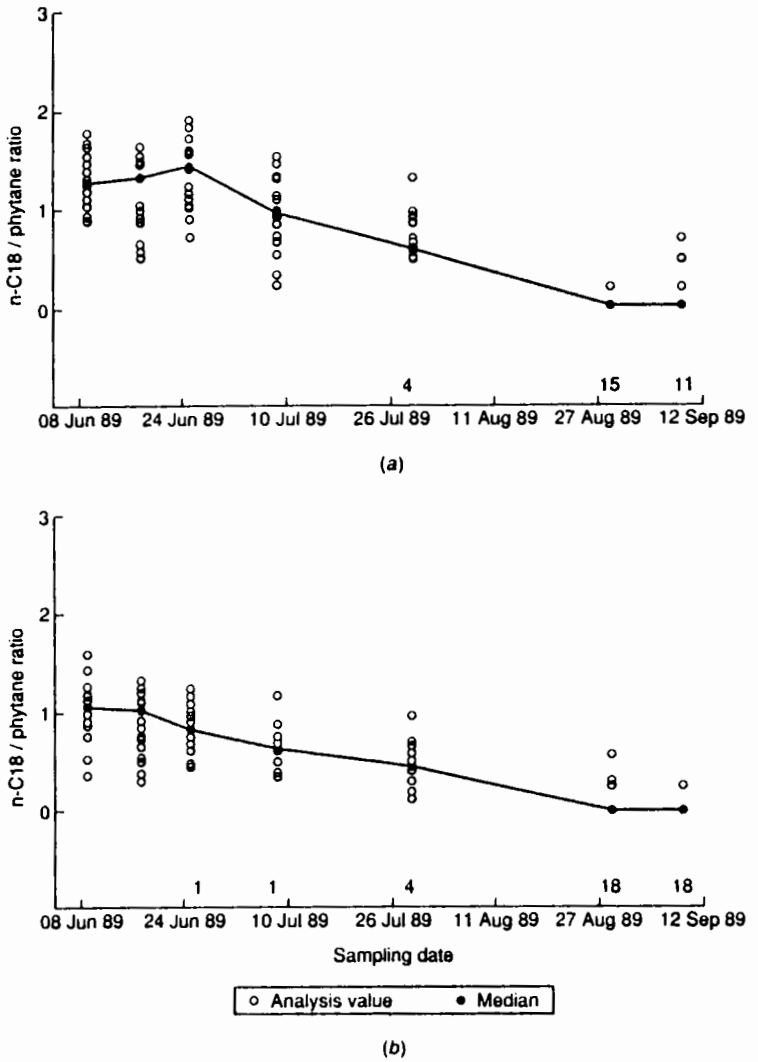


Figure 12.3 Changes in n-C18/phytane relationships over time at treated and control cobble beaches.

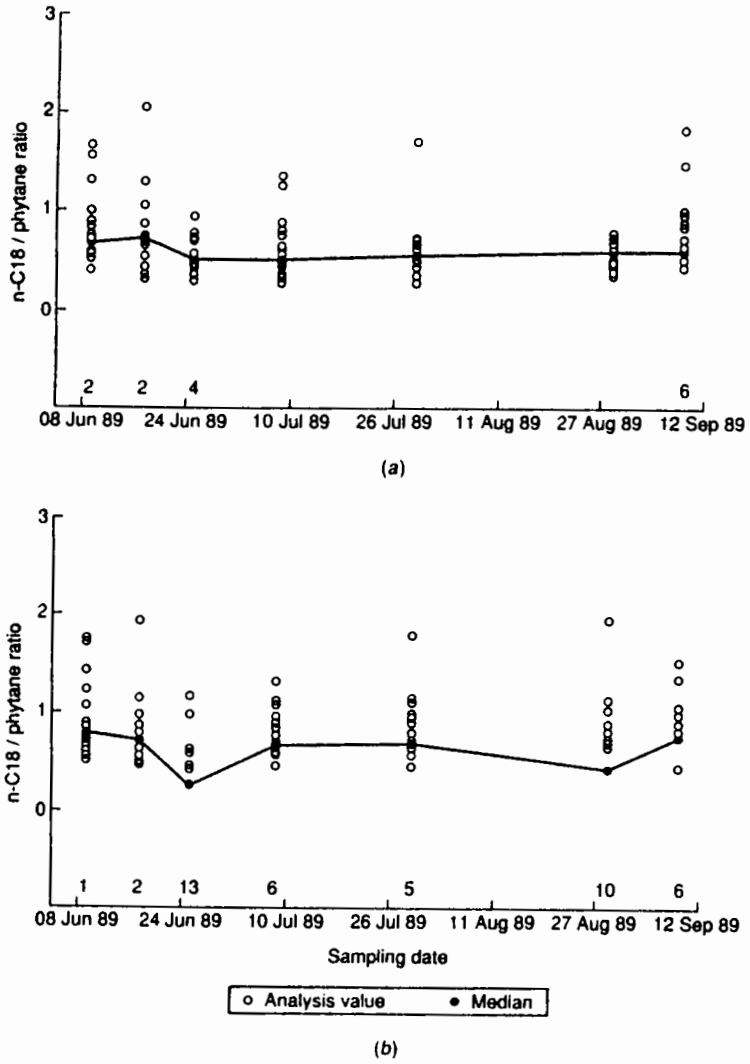


Figure 12.4 Changes in n-C18/phytane relationship over time at treated and control sand and gravel beaches.

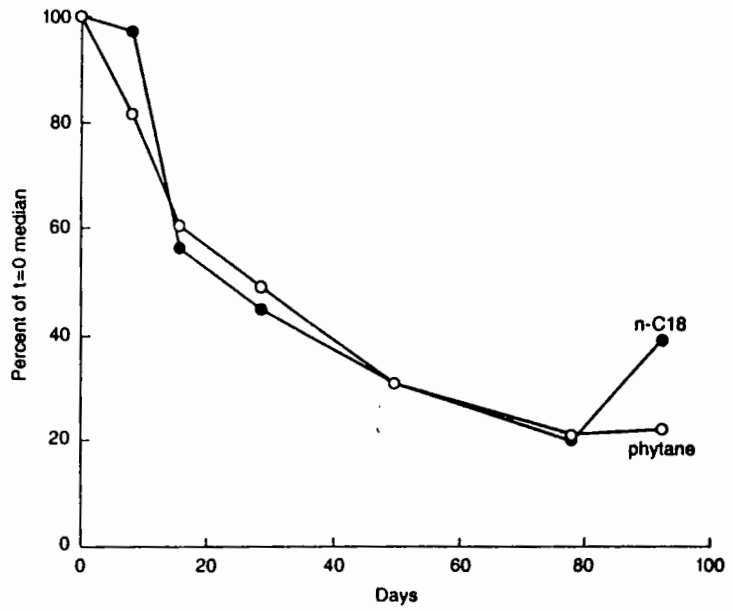


Figure 12.5 Changes in the concentration of phytane and n-C18 (expressed as percent change relative to the t = 0 median concentration) in oil samples from mixed sand and gravel (under the cobble) from Inipol-treated beach plots in Snug Harbor.

have limited subsequent degrees of observable change. However, part of this effect can be explained by another unexpected complication. Examination of phytane itself in the mixed sand and gravel under the cobble on the Inipol-treated beach showed that its decay was as fast as that for n-C18 alkane (Fig. 12.5). Consequently, either biodegradation was not occurring (i.e., some nonbiological process was removing both hydrocarbons simultaneously) or phytane was actually being degraded as fast as the n-C18. Phytane degradation is not common, but it does occur (Pirnik et al., 1977; Mueller et al., 1992), and we have easily isolated phytane-degrading microorganisms from the beach material in Prince William Sound. Thus, microbial communities on Alaskan beaches may have a very pronounced ability to degrade branched alkanes, and the concept of using phytane as an internal biological marker in that case becomes compromised. The cobble surface samples also showed significant decreases in phytane through time, but at a slower rate than the n-C18, thus giving the observed decay in the ratio.

Alternatively, results from the mixed sand and gravel samples under the cobble suggested that possibly Inipol was acting in a chemical man-

ner (surfactant effect) to remove aliphatic and branched hydrocarbons. However, n-C18 and phytane disappeared at essentially the same rate in mixed sand and gravel samples from the untreated control beach (data not shown). Since there was no possibility of a chemical effect on the control plot, one would have to conclude that phytane removal was primarily due to biodegradation.

Compositional changes in aromatic hydrocarbons

At this point we can conclude that biodegradation of the aliphatic fractions of oil was occurring on the samples taken from the cobble surface, and, quite possibly, in the mixed sand and gravel samples as well. As stated above, we believe that biodegradation of the aliphatic fraction was not sufficient in itself to establish that bioremediation was effective. Thus, another approach for examining the overall biodegradation of the oil was to perform selective mass ion spectrometry following gas chromatographic analysis. A variety of aromatic hydrocarbons, which are perhaps more difficult to degrade than the aliphatic hydrocarbons, can be examined with this method (Kennicutt, 1988; Rowland et al., 1986). This is important not only because it tracks another degradable fraction of the oil, but because certain polycyclic aromatic hydrocarbons (PAHs) are known to be procarcinogens under specific conditions. Observing their removal from the oil would therefore imply a reduction in potential adverse ecological effects. Whether this toxicity issue is really relevant (due to improbable exposure scenarios), it was nonetheless a factor to be considered in effectiveness assessments. Furthermore, as the low solubility of the PAHs makes them difficult to degrade, they can be used as the measure of extensiveness of oil degradation. Mass spectral analysis of a variety of aromatic and heterocyclic hydrocarbons in several samples of oil with greatly reduced n-C18/phytane ratios and aliphatic hydrocarbon concentrations is shown in Table 12.4. The selected aromatic hydrocarbons represent a group of methyl-substituted homologs that are found close to the mass number of each parent chemical structure (based on known standards). The values in the table are normalized to hopane (17 alpha, 21 beta), a multiring cyclic alkane (C30). Hopane and its homologs, which are quite resistant to biological attack, have been used for some time as conserved internal biomarkers in oil by the geochemists (Kennicutt, 1988). However, unlike the n-C18/phytane ratio, the relative changes cannot be attributed to biodegradation with as much confidence; differential decay between a hydrocarbon and hopane could be due to nonbiological processes since there may be considerably less chemical similarity between the target hydrocarbon and hopane. Nonetheless, hopane

TABLE 12.4 Relative Concentrations (Mean* and Standard Deviation) of Aromatic, Heterocyclic, and Cyclic Hydrocarbons Normalized to Hopane

	Prudhoe Bay crude	Unfertilized beach	Fertilized beach
n-C18	52.9	1.14 (1.43)	0.96 (0.78)
Phytane	28.3	13.80 (2.30)	6.63 (3.63)
C3/Naphthalenes	31.9	0.15 (0.13)	0.08 (0.04)
C3/Fluorenes	5.30	1.74 (0.38)	1.01 (0.89)
C3/Phenanthrenes	10.0	5.40 (0.25)	3.36 (1.35)
Dibenzothiophene	5.93	0.07 (0.03)	0.04 (0.03)
C3/Dibenzothiophene	9.49	5.34 (0.64)	3.42 (1.43)
Chrysenes	1.22	0.89 (0.04)	0.71 (0.17)
C3/Chrysenes	2.49	1.13 (0.22)	1.03 (0.44)
Norhopane	0.56	0.62 (0.93)	0.59 (0.06)
Stearanes	5.5	5.91 (0.50)	5.15 (0.42)

*N = 8; randomly selected from the 7/29/89 sampling; all samples 50 milligrams of extracted oil residue per milliliter.

provides a consistent standard to normalize the concentrations of aromatic hydrocarbons.

From Table 12.4, the relative difference in the amount of each group of homologs between the beach samples and Prudhoe Bay crude oil indicates the degree of compositional change that occurred in the samples on the beach. Samples from the Inipol-treated beach with very low n-C18/phytane ratios also showed large changes in many of the aromatic hydrocarbons. Norhopane and stearanes are equally as resistant to biodegradation as hopane, and their consistency throughout strongly supports the concept of hopane as a conserved internal biological marker. Again, there was no way to explicitly state that the changes in the aromatic and heterocyclic hydrocarbons were due to biodegradation, but the suggestion is strong. In fact, many of the higher-molecular-weight PAHs are unlikely to be affected by chemical or physical processes, and therefore biodegradation may be the only mechanism that might explain their disappearance. Also, these samples were taken from the beach approximately 78 days after the application of the Inipol fertilizer; any residual chemical effect from the fertilizer that could cause these changes in composition was equally unlikely.

If we assume that nonbiological fate processes (such as leaching or dissolution) remove groups of aromatic hydrocarbons in the order of their solubility, the most soluble being removed first, then any exception to that trend could be attributed to biodegradation since solubility is not the sole characteristic determining susceptibility to microbial attack. Examination of Table 12.4 shows that there are several cases

where decreases in aromatic hydrocarbon concentrations in the field samples (relative to Prudhoe Bay crude oil) are greater for hydrocarbons that are more insoluble. For example, there was a greater decrease in C3-chrysenes than there was in chrysene itself, and there was more decrease in the C3-fluorenes than the C3-phenanthrenes. Although these differences could be an artifact of the sampling and/or analysis, it can be argued that biodegradation was the only process where this differential effect is a possibility. Thus, in most samples where substantial degradation of the oil has occurred (as measured by the n-C18/phytane ratios), there was also a concomitant decrease in many high-molecular-weight aromatic hydrocarbons.

Relationship of compositional change to residue weight change

The next step was to establish a relationship between compositional changes and the loss of oil residues. A positive correlation supports the idea that loss of oil residues was due to biodegradation processes. Figure 12.6 shows a plot of the changes in residue weight against changes in the n-C18/phytane ratio. A good positive correlation was ap-

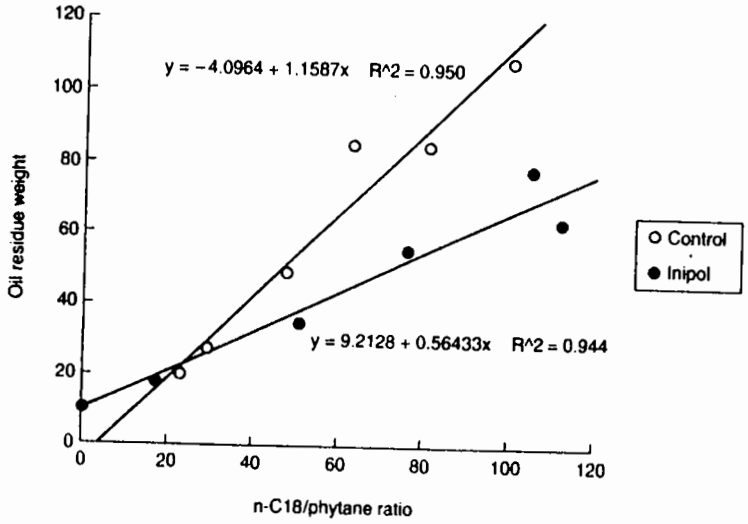


Figure 12.6 Relationship of changes in oil residue weight to changes in the n-C18/phytane ratio (expressed as percent of the median value at t = 0) from oil samples taken from cobble surfaces for both Inipol-treated and untreated beach plots in Snug Harbor. Data points at the lower left corner of the graph represent samples taken late in the sampling period.

TABLE 12.5 Rate Analysis of Natural Log–Transformed Oil Residue Weights (mg/g) in Cobble Surface Samples (July 8, 1989 to July 29, 1989 only) for Test Beaches at Snug Harbor

Beach	Slope (std. dev.)	Significance of slope, greater than zero: N	T-value	P*	Half-life, days	Time to remove 90%, days
Inipol treated	−0.016 (0.007)	80	−2.4	0.02	44	146
Untreated control	−0.006 (0.010)	65	−0.56	0.58	124	411

*Only the Inipol rate is significantly different from zero at the 95% confidence level.

parent. The results are interesting because a rapid reduction in the ratio could occur without a significant change in oil residue weight. However, this was not the case, and we can conclude that once degradation of the aliphatic fraction commences, so does biodegradation of many of the other fractions of the oil, at different rates.

Good quantitative information on biodegradation can now be obtained. Since decay rates for the oil residues appeared to be first order, half-lives of the oil can be calculated (Table 12.5). Application of oleophilic fertilizer caused a greater than twofold increase in the disappearance of oil residues on the cobble surfaces as compared to the untreated control. The difference was statistically significant despite the variability in the data. No difference in the oil residue decay rates was detected in the mixed sand and gravel.

Based on the discussion above we would attribute the greater rate of decay on the fertilizer-treated beach to an enhancement of biodegradation from the provision of nitrogen and phosphorus nutrients. Interestingly, the enhancement effect of the fertilizer appeared to be sustained for as long as 90 days. This time period was well beyond that in which nutrients would be released or in which the fertilizer might have a chemical washing effect. Thus, "priming" the biodegradation process with a little bit of nutrient seemed to go a long way. One can generalize and say that over a 120-day period (i.e., the maximum window for Alaska in which water temperatures are >10°C and thereby adequate for oil biodegradation), bioremediation would remove (assuming linearity) approximately 4 times more oil from the cobble surface than would disappear on untreated control beach. Thus with an initial concentration of 1.0 milligram of oil per gram of beach material (cobble surface), biodegradation can potentially remove most of the oil in a single summer season. This was consistent with our visual observations. The absence of any effect on oil residues in the mixed sand and gravel under the cobble suggested that oil may not have been spread in

a thin enough layer over the beach material to allow bioremediation to have an effect during this testing period. Or possibly the Inipol was unable to provide nutrients to this area of the beach—i.e., it was primarily acting at the beach surface.

Nutrients and microbial biomass

Following the application of the oleophilic fertilizer, interstitial water samples were taken during several tidal cycles to determine if increased concentrations of nitrogen and phosphorus could be observed. Water samples were taken using a modified root feeder apparatus which sampled water 10–15 cm below the surface of the mixed sand and gravel. Sampling was conducted 2, 10, and 30 days after fertilizer application. Elevated nitrogen concentrations were seen only in the day 2 sampling (Table 12.6), but in areas of the test beach, very high concentrations were observed. However, the variability was quite large with somewhat of a bias toward one side of the treated area. If all of the nitrogen in the fertilizer was released at once into a hypothetical body of water overlying the beach test plot at high tide, one would expect concentrations of approximately 200–300 μm N. Obviously, these concentrations were reached in some areas of the beach. Given that three tidal cycles had occurred prior to this sampling, much of the nitrogen in the Inipol fertilizer was probably released in the first few days. This corresponds with the nutrient release data generated from laboratory studies described above. Thus, the enhancing effect of the oleophilic fertilizer on oil biodegradation may have been the result of an initial pulse of nutrients rather than a sustained concentration of nutrients over extended periods. Other laboratory and field data support this possibility (Pritchard et al., 1991).

Increases in oil biodegradation rates as a result of fertilizer application should also result in increases in the number of oil-degrading bacteria. To determine if this was the case, beach samples (mixed sand and gravel) were analyzed using an MPN (most probable number) procedure (Pritchard et al., 1991) in which changes in the physical consistency of the oil were monitored as an indication of oil biodegradation. There was no significant difference between the control and treated beaches over the 3-month sampling period (data not shown). However, as indicated above, the concentrations of oil degraders were very high to start with, and, with the large variability observed in the data, increases of approximately two orders of magnitude were needed to be significant. In addition, increases in biomass could be obscured by sloughing of the cells or predation by protozoa. Field studies the following summer (1990) were finally able to demonstrate significant increases in hydrocarbon degraders but only in the beach subsurface (Lindstrom et al., 1991).

TABLE 12.6 Ammonia Nitrogen (μM) in Interstitial Water Samples Taken on an Incoming Tide, 2 Days Following Application of Oleophilic Fertilizer on a Cobble Beach in Snug Harbor

	Block*			
	1	3	5	7
High-tide zone	57	300	10	4
Mid-tide zone	410	61	3	6
Low-tide zone	190	3	2	3

*Blocks were 5 m long and 4 m wide running end-to-end parallel to the water line and covering three parallel zones, each 4 m wide running side-to-side up the beach. Blocks 2, 4, and 6 in each zone were not sampled.

Disk Island Field Study

A portion of the northwestern shore of Disk Island (located between Ingot and Knight Islands) was chosen as a study site in the summer of 1990, one year after the oil spill. The study was designed to obtain dose-response information for fertilizer application. Fertilizer granules were selected because it was relatively easy to apply different concentrations of the granules in a controlled manner.

The study site was chosen because it was one of the few remaining large areas with moderately to heavily contaminated beach material that could be reasonably used for experimental purposes. The beach area chosen for study, which had not been through the physical washing process used by Exxon, had a shallow slope with little wave activity. Oil contamination was surface and subsurface and was packed into the mixed sand and gravel beach material more densely than observed on other types of beaches in Prince William Sound.

Different amounts of fertilizer granules were applied to plots as shown in Fig. 12.7. The 100 g/m^2 application rate was the concentration of granules applied on a large scale by Exxon. Prior to the fertilizer application, samples of beach material were homogenized and placed in sampling baskets located in each plot. These sampling baskets were then harvested periodically to determine the effect of the fertilizer on oil biodegradation. The homogenization reduced variability in oil concentrations and therefore greatly simplified sampling efforts.

Changes in the concentration of ammonia following application of the fertilizer granules are shown in Fig. 12.8. These data were obtained from sampling wells that were driven into the beach material to allow sampling of the interstitial water with incoming and outgoing tides. The highest concentrations of ammonia were seen with the highest

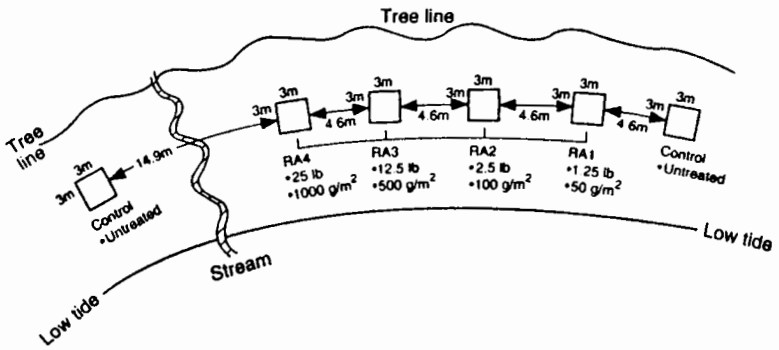


Figure 12.7 Disk Island fertilizer specific activity plot map and rate of CUSTOMBLEN granule application.

concentrations of granules applied (rate 4; 1000 g/m^2), but this concentration was not sustained for more than 1 to 2 days. In fact, ammonia concentrations approached background levels 5 to 10 days after application, regardless of the fertilizer granule concentration applied. Clearly, if a dose response is to be observed, it will result from an initial pulse of nutrients rather than sustained concentrations through time. Results for the release of phosphate and nitrate were similar.

An examination of the decrease in the n-C18/phytane ratio for samples taken from the beaches at different times following initial application of the fertilizer showed that there was essentially no enhancement of biodegradation, as the extent of decrease was not greater than that seen on the control, untreated plots. A comparison of the ratios for a control plot and the plot receiving the highest concentration of fertilizer granules is shown in Fig. 12.9. Biodegradation was obviously occurring (i.e., a steady decrease in the ratios), but it did not seem to be stimulated by the fertilizer. This was a startling result because it reveals that not all beach conditions may be equally amenable to bioremediation. Because of the low-energy features of the beach and the more compact nature of the beach material, mass transport limitations (availability of nutrients and/or oxygen) may have become a significant problem, and this is a key factor to be considered in using bioremediation on other types of oil-contaminated beaches. We are also aware that the beach material contained quantities of humic material; this may have interfered with the oil-degrading microbial communities either as a competing sink for available oxygen or as a degradable carbon source that was preferable to petroleum hydrocarbons.

This experience provides a lesson regarding the use of laboratory tests as an indicator of the potential for bioremediation. If it was a

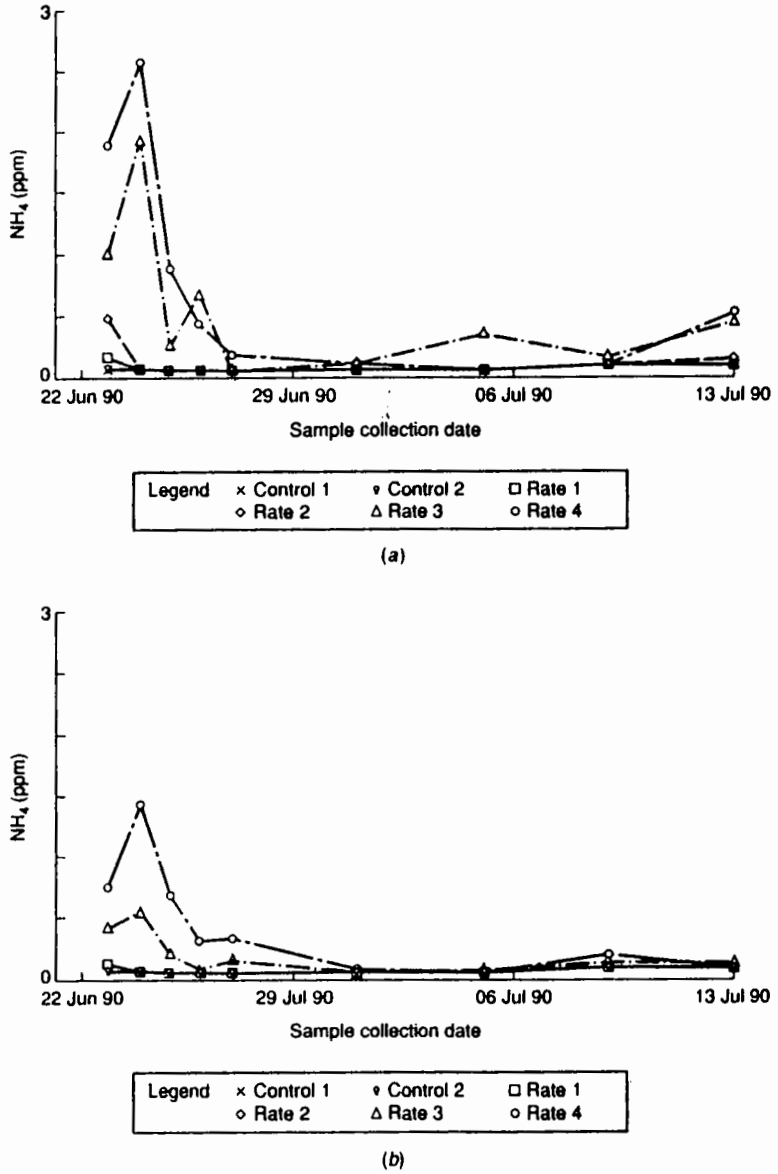
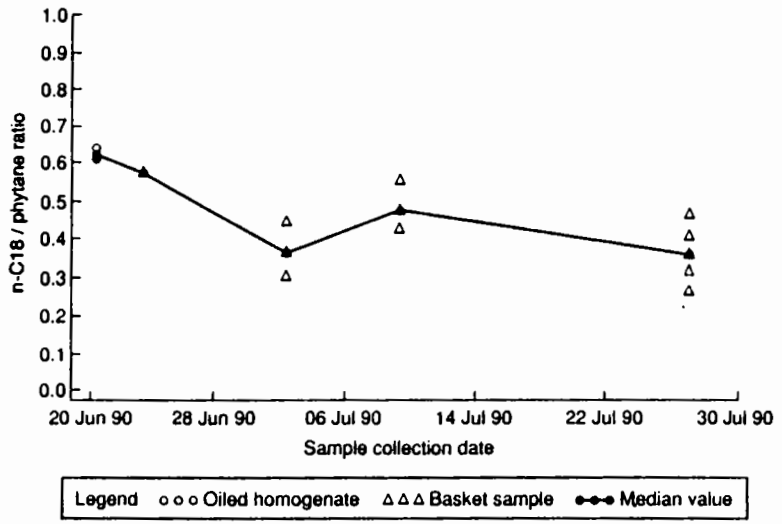
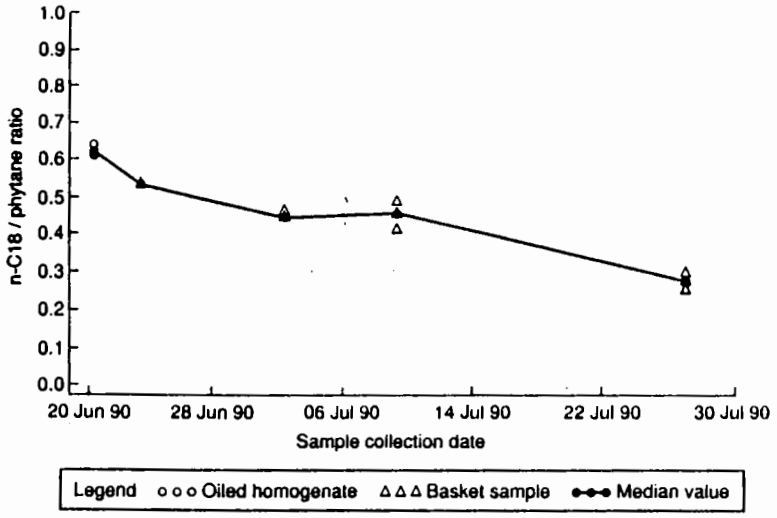


Figure 12.8 (*a*) Changes in ammonia concentration over time for the incoming tide for all plots for the Disk Island Fertilizer Application Rate Study. (*b*) Changes in ammonia concentration over time for the outgoing tide for all plots for the Disk Island Fertilizer Application Rate Study.



(a)



(b)

Figure 12.9 (*a*) Changes in the n-C18/phytane ratio over time for the 500 g/m^2 fertilizer application for the Disk Island Fertilizer Application Rate Study. (*b*) Changes in the n-C18/phytane ratio over time for untreated control plot number 1 for the Disk Island study.

mass transport phenomenon that effected successful bioremediation at Disk Island, removing beach material to the laboratory and conducting tests similar to those described above will likely not reveal the limitations inherent in the field. Most of these laboratory tests involve shake flasks, which by design optimize mass transport, and one would therefore expect that samples may show unrealistically high activities relative to the field. This is illustrated in mineralization studies performed in conjunction with the Disk Island study. Beach material from the sampling baskets, when placed in biometer flasks (see above), showed mineralization activities that reflected an enhancement effect due to the presence of the fertilizer (Fig. 12.10). Some of the total CO_2 production may have been from "nonpetroleum" organic material present in the beach material; however, similar studies using radiolabeled hydrocarbons revealed that stimulation of oil degradation by the fertilizer was probably occurring in these flasks. There also appears to be a dose-response relationship in these results, suggesting that doubling the fertilizer concentrations did not double the oil biodegradation rate as measured by mineralization. Clearly this relationship was not realized in the field.

Thus the stimulatory effect observed in the laboratory was not reflected in the field. The flask studies did, however, indicate that some mass transport limitation was affecting the bioremediation in the field.

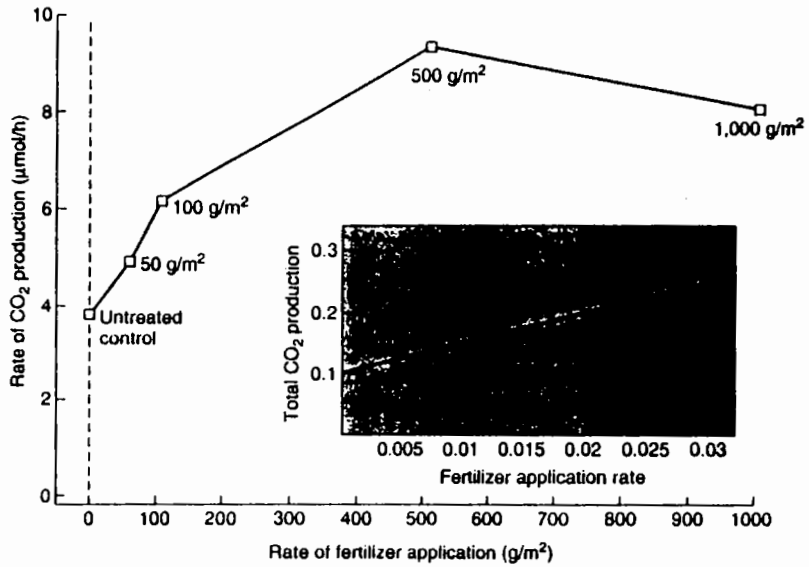


Figure 12.10 Plot of rate of CO_2 production versus rate of fertilizer concentration.

The only way to protect from this extrapolation problem is to perform microcosm studies in which intact samples of beach material can be studied in the laboratory under conditions similar to those in the field. Such systems were in fact developed and tested during the Alaskan oil spill project (Pritchard et al., 1991), but they involve considerably more complexity, time, and expense.

EPA Program in Oil Spill Bioremediation Protocol Development

Closely tied to the field studies just described is the question of which commercial products, whether they are microorganisms, nutrients, surfactants, or others, could or should be used in a bioremediation context. As we mentioned above, in Alaska, many of these commercial products could not be considered because of the very short time frame for field demonstrations, but also because the data available for each product were so variable and/or insufficient that reasonable selections could not be made in a timely fashion. The development of effectiveness and environmental safety testing protocols to be used in establishing a consistent and relevant database, upon which decisions for the use of particular commercial products might be based, is now under way within the Office of Research and Development of the EPA. The conceptual basis for these protocols has proven to be complicated to formulate because of the need to keep the scope of the testing within reason. However, the problems encountered in environmental variability, as illustrated above, make it very difficult to devise protocols that will ultimately provide the "right" kind of information to allow appropriate regulatory decisions to be made. A review of the initial conceptualizing we have carried out to date is in fact quite informative. Obviously, as these protocols are tested and validated, these concepts will have to be modified, so information presented here is not to be considered as final guidelines.

The protocols for determining the effectiveness of oil bioremediation products must ultimately contain the following components:

1. Simplified "expert system" (basically decision trees) for use by regulators that will encourage rational consideration of peripheral factors that are keys to the success of bioremediation on open waters (Tier I—decision trees)
2. A screening test that allows the relative effectiveness of different bioremediation products to be assessed in terms of their ability to promote significant biodegradation of oil under a standard set of laboratory test conditions (Tier II—screening information)

3. A procedure for extrapolating laboratory information to the field on a site-specific basis using definitive kinetic and dose-response information that is integrated with simplified and quantitative predictive frameworks (Tier III—field extrapolation information)
4. A procedure for the use of flow-through microcosm systems to determine the relative effect of different commercial bioremediation products on oil slicks under the environmental conditions that are likely to be experienced during oil spills (Tier III—field extrapolation information)
5. Guidelines for the performance of appropriate controlled field studies in artificial enclosures to clearly establish the fate of the oil during bioremediation (Tier IV—direct field demonstration)

It is prudent to focus on only one portion of the protocol development because of space limitations. I will provide the concept for the testing that would be implemented under the site-specific extrapolation (item 3) and the microcosm testing (item 4) components of the protocol. However, a brief description of the concepts behind a Tier II testing protocol will be given first as a means to develop comparisons.

The purpose of Tier II testing is to determine the ability of a particular bioremediation product to promote significant biodegradation of oil under a standard set of laboratory testing conditions. It is not designed to address effectiveness of a bioremediation product under site-specific conditions.

It is further assumed that the Tier II tests are to be divided into parts: that which measures the activity of biological products and that which examines the effectiveness of nonbiological products. Tests for biological products involve adding the product directly to an oil slick in a proportion recommended by the manufacturer. Other supplements recommended by the manufacturer are also added. It is assumed that the microbial flora of natural water is not required since most of the biological activity is provided by the product. Tests are conducted with and without added nutrients in the water and with sterile (autoclaved) and nonsterile products. Flasks are incubated by shaking at 20°C. If the product is found to be effective, results are compared with a standard data set that is developed from research information specifying the minimum amount of product activity (oxygen uptake profile and changes in oil chemistry) required to make the product effective as a bioremediation agent.

For the testing of nonbiological products, it is assumed that they generally involve some mechanism or procedure for stimulating the oil biodegradation capabilities of natural microbial flora. In most cases this involves the rapid enrichment of the oil-degrading microorganisms

within the total bacterial population. Since at the Tier II level of testing it is not appropriate to consider site-specific factors that affect the activity or enrichment of oil-degrading microorganisms, a standard mixture of bacterial pure cultures that degrade all of the major fractions of the oil tested needs to be used. Alternatively, a specific sampling site can be selected as a consistent source of natural populations of bacteria. However, there is no guarantee that the responsiveness of a water sample from this site will remain constant, and thus the use of a mixture of pure cultures has several advantages: (1) It allows one to store the bacteria over long periods and preserve their activity so that it is the same each time it is used; (2) it eliminates the variances that are likely to occur if natural samples are used as inocula, thereby eliminating the requirement to run reference products each time a test is conducted; (3) optimal conditions of enrichment are employed, thus giving an evaluation of the bioremediation product under ideal conditions. If it does not work under these conditions, then it is very unlikely to work in the field, where the conditions will be a lot less ideal.

Effectiveness of the product will be measured against a standard time, as established by practical conditions at a spill site and by the time it will take to enrich the mixed culture to a point where it will affect the oil. The oil may break up on the surface of the water relatively quickly as a result of the product addition. The test system is designed not only to monitor this event but also, as a closed system, to allow the fate of the oil to be followed for several weeks thereafter. If significant degradation is seen during this incubation period, then it will be assumed that oil leaving the water surface as a result of adding the product will in fact be degraded. Research now carried out under the Oil Spill Research Program will provide a verification for this assumption.

Concept and development of Tier III testing protocols for open water

The appropriateness of oil bioremediation products that are proposed for use in open sea bioremediation must be tested in the laboratory under conditions that are reasonably representative of field conditions. Although tests of this nature can become quite complex, microcosm systems that model special features that might be key to the success of oil bioremediation on open waters have been designed. By far the key feature of the microcosms is the incorporation of a dilution capacity in the microcosms that would simulate the field. Flow-through microcosms that contain an oil slick on the water surface are used in this part of the protocol to assess

1. The tendency of a bioremediation product to remain with the oil long enough to be effective

2. The potential of a product to emulsify the oil and cause it to be dispersed

Microcosm systems that allow the application of a bioremediation product and the necessary supplements to a contained oil surface and then allow seawater to flow under the slick at different velocities and turbulences to create the necessary dilution capacity estimated from a particular field situation will be used. This testing will determine the extent to which the product and the supplements will stay associated with the oil. It will not of course indicate that similar dilution will occur in the field, but it will provide a consistent method to screen, on a relative basis, products and the efficacy of their application strategy. If, for example, a product (or the required supplement) is rapidly removed from the oil slick in the microcosm, it will almost certainly be removed much faster in the field. A product that appears to be effective (i.e., appears to stay with the slick and affect the fate of the oil) would be one considered for further testing. In addition, flow and turbulence conditions in the microcosms can be varied to provide a range of conditions under which the effectiveness of the product could be evaluated.

The Tier III testing is based on biodegradation kinetics and involves the use of microcosm studies to determine the effect of certain key environmental parameters on the ability of the bioremediation product to enhance the biodegradation rates of oil. Kinetic information is very important for protocols dealing with the treatment of oil on the open water because it will always be a question of how fast the product will work under a specific set of environmental conditions *relative* to the rate at which the oil slick is being dispersed naturally.

The use of flow-through microcosms is necessitated by a requirement to determine the effectiveness of a product under conditions that cannot be modeled in a flask study or Tier II level testing. These conditions are defined specifically as the following:

1. Presence of an intact oil layer floating on the surface of a water column
2. Ability to impart and control water column turbulence during testing with the intact oil layer
3. Continuous input of water containing significant concentrations of particulates in the water column
4. Flow-through conditions to allow water exchange and dilution under the oil layer
5. Temperature, particularly as it affects the physical nature of the oil
6. Concentrations of inorganic nutrients in the flow-through water
7. Microbial [illegible] flow water

In the consideration of microcosm testing at the Tier III level, several points must be considered carefully.

1. Microcosm testing is expensive for the vendor because it requires rather elaborate testing facilities, extensive analytical chemical analysis, and sophisticated means of interpreting the resulting data. Thus, the testing should be kept to a minimum.
2. Because of this expense and elaborateness, microcosm testing cannot be used to examine the influence of a large number of ecological or oil spill conditions on the effectiveness of the bioremediation product.
3. Microcosms by definition are designed to simulate certain environmental conditions more realistically than simpler tests, such as shake flasks. Therefore, the specific conditions to be modeled in the microcosm must be critically evaluated such that the microcosm testing does not produce data that is more effectively and efficiently obtained in simpler systems.
4. When considering open-water application of a bioremediation product, it must be realized that modeling conditions typical of a spill site will be very complex, to the point that it is questionable how effectively certain conditions can be appropriately modeled in microcosms. For example, the effectiveness of a bioremediation product will likely be a function, in part, of wave and turbulence conditions. Developing these conditions in a microcosm is difficult. In addition, extrapolation of microcosm information to the field is complicated because these conditions will vary on a day-by-day or even hour-by-hour basis. A range of turbulence/wave conditions could be tested in the microcosm, but this must be limited to testing extremes because of the constraints of cost and time when using microcosms.
5. Finally, it must be kept in mind that Tier III testing involves putting information on the "shelf" to be used at the time of the spill (time is too short following the spill to conduct microcosm-type tests). Thus the shelf data must be of such a nature that it can be used effectively in making decisions at the time of the oil spill.

As mentioned above, one of the most critical factors when performing microcosm studies is the ability to interpret the resulting data. The larger the database available on the product performance, particularly in terms of kinetics, the better one will be able to use the microcosm results and extrapolate the information to a site-specific situation. Because each site is different, it is impossible to have information "on the shelf" for a particular product that will deal with every site. Therefore, one has to make a decision on how to generalize the testing

approach. This can be accomplished either by testing waters from several designated areas (Atlantic Coast versus Pacific Coast or Gulf Coast, northern waters versus southern water, protected bays versus open bays, wetlands versus marshes, etc.) or by examining the effect of selected environmental parameters that encompass, in a general way, all of the conditions in these different areas and then extrapolating the general results to site-specific conditions that can be determined at the time of the spill. The latter approach seems to be the most reasonable.

Consequently, a protocol must be developed based on an initial decision as to the major factors that will most likely affect the performance of the product in treating oil on open waters wherever the spill takes place. In general, the most important factors will be

1. Temperature
2. Turbulence
3. Salinity
4. Background concentration of inorganic nutrients
5. Suspended particulates
6. Background microbial activities
7. Type and concentration of oil

Then it must be determined if the factors can be measured in the field at the time of the spill (within 1 to 2 days). For those that likely can be measured, quantitative relationships can be established between the factor and the product performance. A good example is turbulence. The effectiveness of the product can be established under three different turbulent conditions: high, medium, and low. The data is graphed (effectiveness versus turbulence expressed in Reynolds number or the equivalent), and a relationship established (linear, exponential, etc.) using statistical techniques. Once the relationship is known, then performance of the product for a particular site condition can be predicted. That is, a general indication of turbulent conditions at the spill site will be obtained and the graph will be used to determine the effectiveness of the product.

Since ultimately one will likely be dealing with several environmental factors at once and several specialized environmental conditions, a simple calculation framework can be used. A protocol should stipulate that, for any product, the key environmental factors that will affect performance for any site will be turbulence, temperature, microbial activity, type of oil, nutrient concentrations in the water column, and particulates in the water column. No other factors need be considered as it is assumed that they will be insignificant in the overall decision to use

or not to use the product. Thus every product will have "data on the shelf" that relates these key environmental factors to performance of the product.

Summary and Conclusions

The results from our field demonstration in oil spill bioremediation in Prince William Sound indicate that the oleophilic fertilizer Inipol EAP™ 22 served as an effective nutrient source for oil-degrading microbial communities. It enhanced oil biodegradation, as measured both by changes in oil composition and oil residue weights, by as much as twofold relative to the untreated controls. This was enough of a response to merit incorporation of bioremediation, on a large scale, into the remedial action plan for oil-contaminated beaches in Prince William Sound. Despite this enhancement effect, the importance of its oleophilic nature is still unclear, at least for oil-contaminated beach material from Prince William Sound. However, our studies report the belief that the visual observation of oil removal from the beaches 8 to 10 days following application of the Inipol was due largely to bioremediation and not to a chemical washing effect.

Overall, rapid oil disappearance brought on by the application of the oleophilic fertilizer made these beaches more compatible with local wildlife (less tendency for fur and feathers to become oiled). These changes occurred in a shorter period of time than those limited changes observed in untreated control plots, and possibly helped accelerate biological recovery of the intertidal area.

We have also summarized some of the lessons associated with measuring bioremediation success in the field. Effective measures of biodegradation and interpretation of resulting data, given the highly variable nature of field studies, have been emphasized as a key element in this success. In addition, the complications and difficulties that arose during our use of bioremediation efforts in Alaska were also discussed. Hopefully this will help guide similar applications of bioremediation at future spills.

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. AUTHOR(S) '. H. Pritchard	8. PERFORMING ORGANIZATION REPORT NO.
·. PERFORMING ORGANIZATION NAME AND ADDRESS J.S. Environmental Protection Agency, Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida	10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
i2. SPONSORING AGENCY NAME AND ADDRESS U.S. ENVIRONMENTAL PROTECTION AGENCY ENVIRONMENTAL RESEARCH LABORATORY OFFICE OF RESEARCH AND DEVELOPMENT GULF BREEZE, FLORIDA 32561	13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

In. Biotreatment of Industrial and Hazardous Waste. Morris A. Levin and Michael A. Gealt (ed.), McGraw-Hill Book Co., New York, p. 269-307

16. ABSTRACT

The use of bioremediation as a supplemental cleanup technology in the *Exxon Valdez* oil spill, in Prince William Sound, Alaska, has proven to be a good example of the problems and successes associated with the prac tical application of this technology. Field studies conducted by scientists from the U.S. Environmental Protection Agency have demonstrated that oil degradation by indigenous microflora on the beaches of Prince William Sound could be significnatly accelerated by adding fertilizer directly to the surfaces of oil-contaminated beaches. Our results from the application of an oleophilic fertilizer are presented as exemplary field and laboratory information. The fertilizer enhanced biodegradation of the oil, as measured by changes in oil composition and bulk oil weight per unit of beach material, by approximately twofold relative to untreated controls. These studies supported bioremediation as a useful cleanup alternative that was subsequently used by Exxon on a large scale. They have also generated a number of insightful lessons that have significant relevance to future oil bioremediation efforts. This chapter discusses these lessons and examines complications and difficulties in assessing the effectiveness of bioremediation in the field.

17. KEY WORDS AND DOCUMENT ANALYSIS

A. DESCRIPTORS	B. IDENTIFIERS/OPEN ENDED TERMS	C. COSATI FIELD/GROUP

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (*THIS REPORT*) UNCLASSIFIED	21. NO. OF PAGES 38
RELEASE TO PUBLIC	20. SECURITY CLASS (*THIS PAGE*) UNCLASSIFIED	22. PRICE

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