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Oil spill bioremediation: experiences, lessons and results from 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 was accelerated by adding fertilizer directly to the surfaces of oil-contaminated beaches. Although several types of fertilizers were used in the studies, only the results from the application of an oleophilic fertilizer are presented. The fertilizer enhanced biodegradation of the oil, as measured by changes in hydrocarbon composition and bulk oil weight per unit of beach material, by approximately two-fold relative to untreated controls. Laboratory studies verified the usefulness of the oleophilic fertilizer as a nutrient source, but the contribution of its oleophilic components towards enhancing biodegradation is still unclear.

These studies supported bioremediation as a useful cleanup strategy that was subsequently used by Exxon on a large scale. The *Exxon Valdez* experience has also provided a number of informative lessons that have significant relevance to future oil bioremediation efforts. This paper discusses these lessons and the difficulties in assessing the effectiveness of bioremediation in the field.

Introduction

The potential usefulness of bioremediation to clean soils, sediments and aquifer materials contaminated with oil and petroleum hydrocarbons has been recognized for some time (Morgan & Watinkson 1989; Lee & Levy 1989; Bartha 1986). The recognition of oil as a complex but largely biodegradable mixture of hydrocarbons and the knowledge that hydrocarbon-degraders can be enriched in many, if not most, types of environments (Lee & Levy 1987; Atlas 1981), has contributed greatly to the development of oil bioremediation technologies. In addition, bioremediation is rapidly gaining acceptance as a viable technology and if used prudently, can provide efficient, inexpensive and environmentally safe cleanup of waste chemicals.

The feasibility of bioremediation as a supplemental cleanup tool in the *Exxon Valdez* oil spill, in Prince William Sound, Alaska was timely and scientifically reasonable (Pritchard & Costa 1991). Analysis of data collected from subsequent field studies to determine if oil degradation by indigenous microflora on the beaches of Prince William Sound could be significantly accelerated by fertilizer application (Pritchard et al. 1991) suggested a new and workable remedial action.

A number of useful lessons were learned that will have significant meaning to future oil bioremediation efforts. This paper summarizes some of these lessons and highlights several of the difficulties that arose during the use of bioremediation in Alaska. Results from the application of an oleophilic fertilizer as part of a field demonstration project conducted at Snug Harbor on Knight Island in Prince William Sound will be the primary focus of discussion. We hope this information will stimulate others to carefully consider the process by which bioremediation success is measured in the field and will further emphasize the need to continually expand our research data base, despite the quantity of background information that may already exist.

Background

Any bioremediation effort must invariably involve a scientifically valid demonstration of process effectiveness and environmental safety. Effectiveness, in the case of oil bioremediation, means establishing that: a) the removal or disappearance of the oil is primarily attributable to biodegradation and not other processes, and b) enhanced biodegradation rates of oil are sufficiently faster (with statistical verification, if possible) than natural rates to justify expenditure of effort to implement the bioremediation process on a large scale. Although environmental safety issues are not addressed in this paper, they too require considerable effort to verify that no adverse ecological effects occurred as a result of the fertilizer application.

For the *Exxon Valdez* oil spill, both aspects were crucial to the eventual acceptance of bioremediation by the public and state and federal regulatory agencies. While confidence provided by almost 20 years of accumulated research data on oil biodegradation laid the foundations for our approach in Alaska, the complexity of the filed application and the problems associated with demonstrating successful bioremediation on a large scale were substantial. These aspects are discussed below. Remember that the effectiveness issues addressed in Prince William Sound, where oil-contaminated gravel and cobblestone beaches were treated, will not be the same as those addressed for oil-contaminated sandy beaches, marshes and wetlands.

Enrichments of oil degrading microbial communities

Despite the cold water temperatures of Alaska, significant enrichment of oil degrading microorganisms was expected in the beach material following exposure to the oil. Research by Atlas and his colleagues supported this assumption (Atlas 1981). Approximately two months after the spill, concentrations of oil degraders averaged 10^6 per gram of oiled beach material, representing as much as a 10,000-fold increase in the number of oil degrading microorganisms relative to uncontaminated beaches (Table 1). Studies at a later date by Lindstrom et al. (1991) have showed similar differences. Enrichments of this magnitude suggested the oil was being degraded (previous laboratory studies have demonstrated that Prudhoe Bay crude oil is readily biodegradable), that nitrogen and phosphorus were available to support the growth of the hydrocarbon degraders, and that the cold temperatures (10–16 °C) were probably not overly restrictive to the degradative capabilities of the indigenous microflora. This high biomass, a great excess of degradable organic carbon from the oil, and a finite supply of nitrogen in the water, suggested the potential of nitrogen-limited biodegradation and also opened the possibility of accelerating oil biodegradation by overcoming this limitation.

In this context, it was unnecessary to experimentally reverify the stimulatory effect of nitrogen on oil biodegradation in the laboratory, even using oil-contaminated beach samples from Prince William Sound, since numerous published reports in the literature supported this as a sensible approach. Instead, because of the importance and magnitude of the problem, it was reasonable to go directly to the field and conduct a practical demonstration. Thus, in the very early stages of the oil spill cleanup program in Alaska, the simple measurement of the number of oil degrading microorganisms, along with a wealth of relevant supporting literature, provided an initial indicator of the feasibility of oil bioremediation in this situation.

Other indicators of oil degradation activity

There is a strong tendency, by most decision-making officials involved in oil spill cleanup, to generate 'site-specific' information about bioremediation potential; information from other sites or experiences is often not acceptable or applicable. Circumstances in other spills may dictate that information on the enrichment of oil degraders in the contaminated areas alone is not sufficient for initial decision-making on bioremediation. A further discussion of these indicators, even though they were not used initially in Alaska, is therefore relevant.

For example, mineralization studies involving measurements of total CO_2 production can provide excellent initial information. The approach provides rapid, relatively unequivocal, time-course data suitable for testing different biological 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 compared to a control containing uncontaminated beach material. Likewise, nitrogen or other additives should readily stimulate the biodegradation rate in oil contaminated beach material. Biometer flask systems (Bartha & Pramer 1965), designed to trap CO_2 in side-arms containing an alkaline solution (Fig. 1) can be adapted to measure oil mineralization rates from contaminated beach material (Mueller et al. 1992). 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 simulating tidal exchange by flushing fresh seawater in and out (Mueller et al. 1992). An example of the data generated from such a mineralization test is shown in Fig. 2. This experiment was performed with oil-contaminated Prince William Sound beach material taken after our bioremediation field demonstration had begun. The biometer flask method is indicative of the short term site-specific tests that can be initially conducted as a data gathering exercise to develop cleanup strategies for a particular oil spill. Note the enhancing effect of adding nitrogen fertilizer (Fig. 2). By comparing rates of CO_2 production, an estimation of the extent of enhancement in oil biodegradation can be obtained. The system can be easily adapted to test beach material from other oil spill sites.

Table 1. Relative concentration[a] (Log_{10} of the cell numbers/g of beach material and standard deviation) of oil-degrading microorganisms in samples from beaches exposed and unexposed to oil.

Unexposed sites	High tide	Mid tide	Low tide
Tatitlek	2.41 (±0.58)	4.31 (±1.14)	6.11 (±2.05)
Snug Corner Cove	2.31 (±0.54)	2.51 (±0.55)	<2.1
Hell's Hole	<2.1	2.51 (±0.89)	<2.1
Commander Cove	4.51 (±1.14)	<2.1	3.11 (±0.45)
Oil exposed site	**Eagle Beach**	**Otter Beach[b]**	**Seal Beach[b]**
Snug Harbor[c]	5.18 (±0.91)	5.75 (±1.29)	5.65 (±1.04)

[a] Based on most probable number determination.
[b] Prior to fertilizer application.
[c] Mid tide zone.

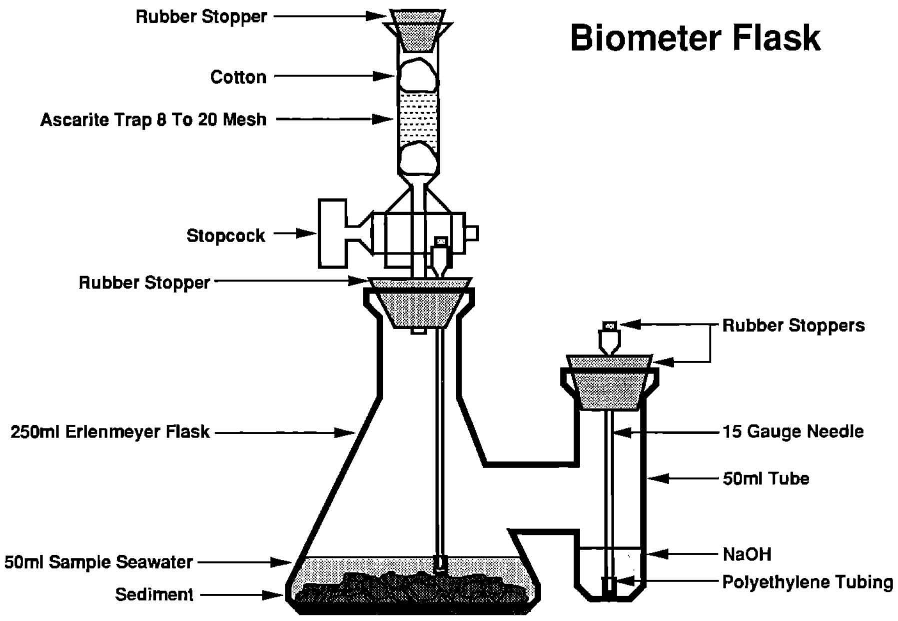

Fig. 1. Modified biometer flasks used to measure mineralization of oil from contaminated beach samples taken directly from the field.

Since any other forms of organic matter in the beach material can also produce CO_2, care must be taken to assure that one is measuring only oil 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 radiolabelled hydrocarbon. If oil is actively degraded (with or without nitrogenous nutrients added), the production of radiolabelled CO_2 should be extensive and immediate. Results have demonstrated that phenanthrene worked well as an indicator because it was rapidly and completely sorbed to the oil (Mueller et al. 1992; Pritchard et al. 1991). Since the tidal cycle washed out any residual aqueous-phase phenanthrene, we assume that CO_2 production was mostly from the bacterial communities associated with oiled surfaces.

Oil spill bioremediation as a finishing step

We assumed that bioremediation, for more heavily contaminated areas, would be most effective as a finishing step, following some physical measure to remove bulk oil. Clearly, the time required for cleanup depends on the mass of oil present; since oil degradation occurs primarily at the water interface, the more oil, the longer it will take to be degraded. In Alaska, where the treatment window for active oil biodegradation was limited to the summer months when highest water temperatures occurred, the time required for successful bioremediation was particularly critical. In other oil spill situations, more time may be available for cleanup and therefore, consideration of oil bioremediation as a primary cleanup method may be reasonable. Greater oil mass, however, will undoubtedly require some procedure for maximizing the interaction between the oil degrading microbial communities and the oil, such as by mechanical tilling.

The physical washing procedure employed by Exxon in the Alaskan spill removed bulk oil, but the beach material remained quite contaminated, aesthetically unpleasing and the oil residues ecologically available. As there were very few follow up treatment alternatives, bioremediation was appealing. However, without the initial removal of this bulk oil, bioremediation may not have been practical in Alaska because even if oil biodegradation was quite active, there was only a limited time

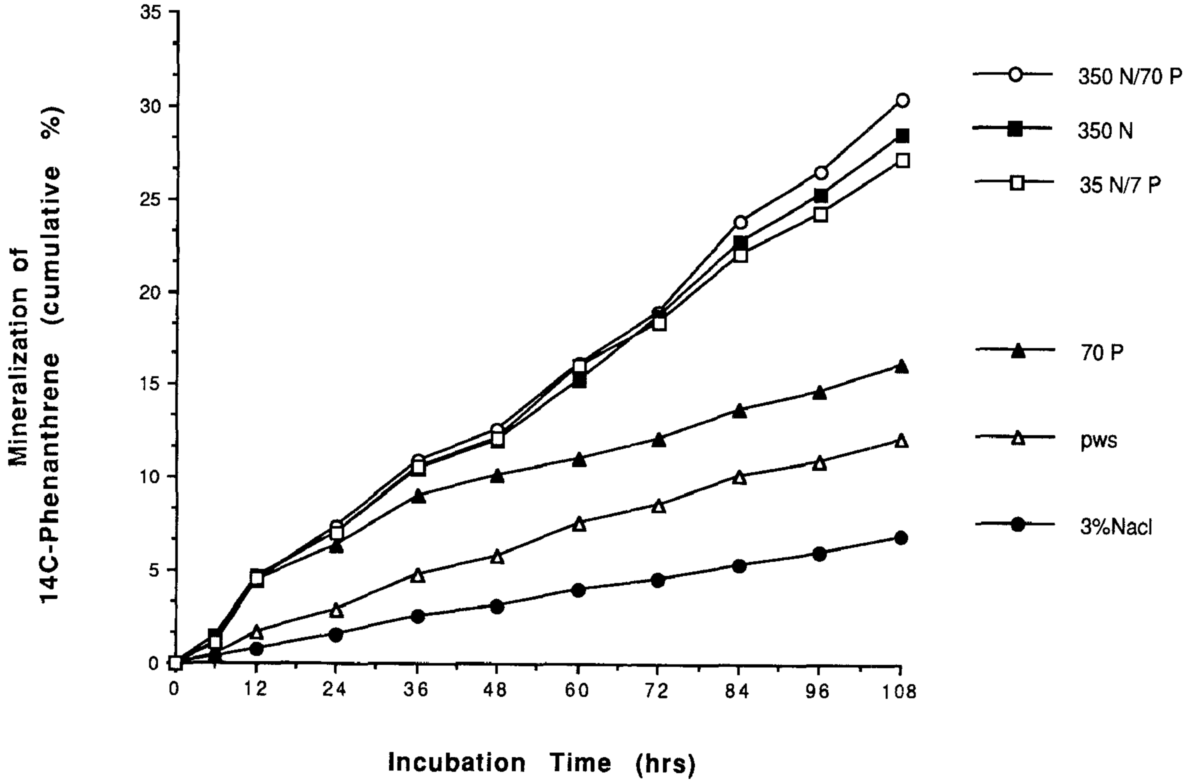

Fig. 2. Cumulative CO_2 production from ^{14}C-phenanthrene added to oil-contaminated beach samples taken from Prince William Sound and as affected by the addition of different concentrations of phosphorus (P, 70 ppm) and nitrogen (N, 35 and 350 ppm). Nutrients were added as inorganic salts (sodium phosphate and ammonium chloride) dissolved in Prince William Sound (PWS) seawater.

to degrade oil to any significant depth. Thus, for more short-term results, the oil must first be further distributed throughout the beach material to increase the surface to volume ratio. Since surface cultivation (i.e., tilling) was unreasonable on most of the cobblestone beaches in Alaska, effective bioremediation depended, in part, on bulk oil removal by the physical washing process and the consequential dispersion of residual oil into the beach material. However, the physical washing may have forced oil to a greater depth in the beach material and possibly affected its ultimate biodegradation or bioremediation.

The highly porous beaches of Prince William Sound were advantageous for bioremediation; oil that diffused into the gravel was spread over a much greater surface area and the oil degrading bacteria were continually bathed at each high tide with oxygenated water. On a sand or mud shoreline, where porosity is much lower, most of the oil will probably be located at the surface. However, the contaminated beach material can be physically removed and the remaining contaminated beach material can potentially be treated by bioremediation, as a finishing step. Tilling may be used to further disperse the remaining oil into the beach material, increasing the surface to volume ratio. Tilling also aerates beach material and helps disseminate added fertilizers.

Choice of fertilizer formulations

We assumed for Alaskan beaches that nitrogen (and, to a lesser degree, phosphorus) had to be applied in a manner that would passively expose the oil degrading microbial communities to the elevated nutrient concentrations over an extended time. Due to the large tidal volume and significant wave action, soluble or easily dissolved fertilizer materials placed on the beach surface would likely wash away in a few days. To avoid this problem, two types of slow release fertilizers were initially considered; solid pelletized formulations and liquid oleophilic formulations. Characteristics of each fertilizer selected are summarized in Table 2.

Although we will not present data here on the effectiveness of the solid fertilizer formulations in bioremediation, briefly summarizing some of the criteria used to make the fertilizer selections is instructive, as this could help in making fertilizer selections in the future. The three main criteria were a) ease of application and potential to retain position on the beaches, b) nutrient release characteristics, and c) 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 adhere tightly to the oiled beach material and worked their way down under the cobble where they were difficult to dislodge. Nutrient release characteristics, which were determined in simple laboratory test systems (Safferman 1992; Glaser et al. 1991), showed that much of the nitrogen (ammonia and nitrate) and phosphorus (phosphate) released from the granules occurred in the first 24–72 hours. However, sufficient quantities continued to be released steadily for a considerable time thereafter. Thus, as the tides washed the beach, nutrients were distributed to the microbial communities associated with the oil for a period of 2 weeks or longer. Although the physical condition of the granules slowly deteriorated on contact with seawater, they persisted on the beaches, with fertilizer inside, for 2–3 weeks after application. The fertilizer granules were ultimately used by Exxon in combination with the liquid oleophilic fertilizer for much of the large scale applications in Prince William Sound.

Fertilizer briquettes were also found to be satisfactory based on the above criteria (Safferman 1992). The briquettes are approximately the size of charcoal briquettes and contained organic sources of nitrogen and phosphorus that slowly hydrolyzed to release urea and phosphate. Nutrient release characteristics of the briquettes were very similar to the granules. Although the briquettes maintained their physical integrity, if broadcast over the

Table 2. Description of fertilizers tested.[a]

Commercial name	Woodace	Customblen	Inipol
Manufacturer	Vigoro Industries	Sierra Chemical Co.	Elf Aquataine
Form	Briquette	Granule	liquid
Size	5 × 5 × 5 cm	2–3 mm dia	–
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 gm/ml
Viscosity	–	–	250 cst
Application rate in 12 × 35 m plots	986 gm/m^2	100 g/m^2	284 gm/m^2
Method of application	Net bags (11.8 kg ea.)	Fertilizer spreader	Back pack sprayer
Test areas	Snug Harbor	Snug Harbor Passage Cove	Snug Harbor Passage Cove

[a] Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

beach surface, they were easily moved around by the tides and waves, resulting in heterogenous distributions. Consequently, they had to be packaged in net bags and secured to the beach with metal stakes. Although this was effective, it presented significant logistical problems precluding their consideration for large scale use. The briquettes were, however, used as part of our initial field demonstration in bioremediation and appeared effective in enhancing oil biodegradation (Pritchard et al. 1991).

In consideration of future applications, rather than broadcast fertilizer granules and briquettes on to the beach surface, they could also be buried in the beach material, e.g. 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, nutrients could be effectively distributed 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 tested, because of potential logistical restrictions and vigorous storm action. 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) 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. 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, was to apply as much as possible without exceeding toxic concentrations of ammonia and/or nitrate. Ammonia and/or nitrate are toxic (96-hr LC50 = 10–15 ppm) to mysid shrimp, a good sentinel for most sensitive species (Pritchard et al. 1991). Given the pulsed nature of nutrient release from these fertilizer formulation, concentrations of applied ammonia/nitrate could exceed toxic concentrations and still be acceptable if dilution quickly reduced the concentrations. How much the toxic concentration can be exceeded will depend on the dilution capacity in the area of application and data from laboratory toxicity tests using pulsed exposure to the nutrients.

Alternatively, fertilizer application rates could be determined using information on the amount of nutrients required to metabolize a given quantity of oil. Since this approach will depend on the amount of oil treated and the C:N ratios for optimal oil biodegradation (Morgan & Watkinson 1989), it is likely to be a complicated assessment due to the heterogeneity of oil distribution invariably encountered in the field and the impact of tidal flux and dilution.

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 adhere to the oil, keeping 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 & 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 (2-ethylhexyl-dipolyethylene oxide phosphate) (Bergstein & Vestal 1978), for several natural sources of lipophilic nitrogen and phosphorous, 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 & Levy 1987; Halmo 1985). Therefore, the advantage of using oleophilic fertilizers has not been clearly established. None the less, 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.

The oleophilic fertilizer selected was Inipol EAP 22, produced by Elf Aquataine Company in France, since it was the only commercially available source with large production capability. This unique product is a stable micro-emulsion consisting of a 'core' of urea (the nitrogen source) surrounded by an oleic acid carrier. Tri(laureth-4) phosphate (a surfactant and the source of phosphorus) acts as an emulsion stabilizer and butoxy ethanol reduces viscosity. Inipol EAP 22 has shown promise in tests conducted in the laboratory and in large outdoor tanks using different types of oil-contaminated beach materials and different environmental locations (Lee & Levy 1987; Sveum & Ladousse 1989; Tramier & Sirvins 1983). Interesting results were obtained from a test conducted on an oil-contaminated beach in Spitsbergen, Norway (Sveum & Ladousse 1987). Inipol appeared to increase oil biodegradation rates when applied to coarse-grained, gravely 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% of the urea (measured as total Kjeldahl nitrogen, TKN) was released within the first few minutes after application (Safferman 1992). Measurements of total phosphorus showed a similar release pattern. A 6-hour wait before the first submersion produced the same results as a 5-minute wait, suggesting that the Inipol 'sets' quite quickly. However, following this initial burst of TKN and phosphorus, there was essentially no release of these materials thereafter. Presumably, the residual nitrogen and phosphorus, although tightly held to the oiled beach material, was available to the bacteria degrading the oil. Experimentally demonstrating this availability was difficult and is 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). Coatings were controlled visually 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. Manufacturers recommended application rate (5% of the oil) could not be followed because oil concentrations were highly variable. In the large scale use of Inipol by Exxon, an application rate of approximately 0.3 liter/m^2 was used, based, in large part, on simulating 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 in bioremediation, produced some surprising visual results (Pritchard & 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 because the concentration and distribution of oil was thought to simulate physically-washed beaches. Visually, the cobble areas had a thin coating of sticky oil covering the rock surfaces and mixed sand and gravel under the cobble. Oil penetration was < 0.3 cm below the surface. In some areas, small patches of thick oil and mousse could be found.

Approximately 10–14 days following oleophilic fertilizer application, reductions in the amount of oil on rock surfaces were visually apparent. It was particularly evident from aerial observations, where the contrast with oiled areas surrounding the plot was dramatic, etching a 'clean' rectangle (area where the Inipol was applied) on the beach surface (Fig. 3). The contrast was also impressive at ground level; there was a precise demarkation between fertilizer-treated and untreated areas. At this time, the untreated control plots appeared unaltered visually.

Fig. 3. Visual effect of Inipol fertilizer application on the removal of oil from test plots in Snug Harbor on Knight Island, Prince William Sound. Fertilizer treated area is shown on the right.

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, but with no tendency to come off the rocks. Concurrently monitoring of the shore line waters revealed that no oil slicks or oily materials were found leaving the beach during tidal flushing.

The visual disappearance of oil on the cobble surface 2–3 weeks following Inipol application was largely due to biodegradation and not a chemical washing phenomena. Supporting chemical data are presented below. When large concentrations of Inipol were applied repeatedly on several mini plots in Snug Harbor to maximize any potential chemical washing effects, oil removal was not affected; that is a period of at least 2–3 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 rather than chemical washing (Pritchard et al. 1991). Finally, laboratory tests performed by Exxon researchers (R. Prince, S. Hinton, and J. Bragg, personal communications) have shown that Inipol, when compared to chemicals specifically designed as rock washers, was ineffective. Also, they observed that in microcosm studies Inipol caused the oil to become more tightly associated with the beach material; that is, in the absence of Inipol, the oil had much more of a tendency to move to the glass walls of the microcosms.

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, contributed to these changes.

Since the disappearance of oil on the untreated control beaches eventually 'caught up' to that obtained on the treated beach, it can be argued that the cost and effort of bioremediation may not have been justified. However, rapid oil disappearance, caused by the application of the oleophilic fertilizer, potentially made these beaches safer for local wildlife (less tendency for fur and feathers to become oiled) in a shorter period of time, a highly desirable situation where breeding and migratory activities are prominent as they were in Alaska. Depending on the time of season, accommodation of these activities could not have been realized by 'doing nothing' or by just performing physical washing. On beaches more heavily oiled than our test plots in Snug Harbor, natural biodegradation would not likely remove all of the oil before the winter season curtailed this degradative activity. Accelerating the degradation through the application of fertilizer during the active summer months undoubtedly reduced the need for further cleanup and limited the potential of ecological exposure to the oil the following spring. Finally, the more quickly oil can be removed from a beach, the more rapidly recolonization of those animals and plants killed initially by the oil spill can occur. Consequently, bioremediation could help accelerate biological recovery of the intertidal zones during times when biological activities were at their peak.

Measures of oil spill bioremediation effectiveness

Obtaining definitive information on the role of biodegradation in the removal of oil residues from beach material, or from any complex environmental matrix, is a difficult task. In general, to assess the effectiveness of oil spill bioremediation, information must be produced on (1) qualitative changes in hydrocarbon composition that are indicative of biological processes and (2) decay rates of bulk oil 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, branched hydrocarbons, polar chemicals, etc.), is desirable.

Simultaneously, the quantitative information can establish that the enhancement of oil biodegradation by the fertilizer treatments was sufficient to merit full-scale operation; generally a 2- to 3-fold enhancement over the untreated controls might be acceptable scientifically and probably to many decision makers and regulatory groups, but this is not based on a comprehensive data base. Both types of information, however, were difficult to obtain in the field.

Any bioremediation testing program that is based on analytical techniques involving decreases of oil residues or hydrocarbons resolvable by gas chromatography is open to scientific criticism because other environmental fate processes (including photolysis, physical dissolution, chemical washing, volatility, etc.) can affect or contribute to the disappearance of oil. To confront these potential criticisms, we chose an approach that integrated several analytical procedures with several key assumptions. The first assumption was that the disappearance of several target hydrocarbon groups could be used as definitive indicators of biodegradation. Furthermore, strong indications of biodegradation would be associated with substantial changes in the composition of other fractions in the oil, particularly selected aromatic hydrocarbons.

The second key assumption was 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 information could then be used to estimate cleanup effectiveness over extended time periods.

Some discussion as to why this approach was selected is in order because the criteria as to what constitutes biodegradation of oil is complicated and controversial. Many studies have considered measurements of reductions in aliphatic hydrocarbons concentrations as generally indicative of overall biodegradation but the value of such reductions is often questioned because these hydrocarbons are: a) frequently the most readily degradable fraction, b) the least toxic and c) often only a small percentage (by weight) of the oil. Measuring compositional changes in the aromatic fraction adds a further dimension to biodegradation, as these hydrocarbons are less readily degradable and potentially cause chronic toxicity. Common procedures for measuring the aromatic hydrocarbons are usually based on mass spectral analysis which concentrates on only 10–20 selected compounds and represents only a very small fraction of the total aromatics. Whether these selected aromatic hydrocarbons are reasonable surrogates for the degradation of the rest of the aromatic compounds has not been established. However, if the aromatic hydrocarbons are shown to degrade, aliphatic hydrocarbons will almost certainly be extensively degraded as well.

Regardless of compositional changes, it seems intuitive that bioremediation should effect the removal of bulk material as well; changes in hydrocarbon composition without much change in oil residue removal seems to present only part of the picture. Moreover, oil biodegradation under optimized conditions in the laboratory will result in as much as a 40–60% reduction in the total weight of oil (Atlas & Bartha 1973). Therefore, some reduction in oil residue weight should be reasonably 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 for each of these fractions. These fractions can be further 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 composition

Results with the oleophilic fertilizer provide perspective on measuring effectiveness in bioremediation at a field demonstration level. 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, seven 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 (Pritchard et al. 1991). It is assumed that all areas of the treated beach were equally exposed to the fertilizer, although there was no certainty in this respect. The weight of oil recovered on a mg/g basis of beach material was determined gravimetrically and hydrocarbon composition was determined by gas chromatography of the extracts following standard analytical procedures.

Changes in composition were determined first by examining the resolvable alkanes. Historically, this is done by calculating the weight ratio between a hydrocarbon that is known to readily biodegrade (generally the C-17 and C-18 normal alkanes) and ones that are slower to biodegrade, such as the branched alkanes, pristane and phytane, which elute closely to the n-C17 and n-C18 alkanes, respectively (Kennicutt 1988). We focused on the n-C18/phytane ratio because pristane is sometimes found naturally in seawater (C. Henry, pers. comm.). The ratio concept is based on the idea that most nonbiological fate processes (physical weathering, volatilization, leaching, etc.) will not produce differential losses of normal and branched hydrocarbons that have similar gas chromatographic and correspondingly, chemical behavior. Support for this concept can be found in biogeochemical studies on oil (Kennicutt 1988).

The technique has two disadvantages. First, since the branched alkanes do, in fact, biodegrade (Prinik et al. 1977; Mueller et al. 1992), they need only degrade more slowly than the straight chain alkane to obfuscate the ratio method resulting in a very conservative measure of biodegradation. Second, octadecane is likely to be one of the first alkanes degraded, making the ratio technique useful only in the early stages of oil degradation. Also, because the alkanes may not make up a large percentage of the crude oil (6% in Prudhoe Bay Crude), the change in ratios may not be indicative of significant or longer-term changes in other components of the oil.

The effects of the oleophilic fertilizer, Inipol EAP 22, on changes in alkane and branched alkane composition in samples from the cobble surface, are shown in Fig. 4A and B. Extensive decay in the n-C18/phytane ratio occurred through time. The decay rate for the fertilizer-treated beach was slightly faster; the difference in the slope of linear regression lines was approximately 1.2 but was not statistically significant ($p = 0.05$). However, comparing the slopes of these decay curves is complicated by the great heterogeneity of the samples and because of the apparent lag observed on the Inipol-treated beach. The cause of this lag is not known but it does not appear to be Inipol specific, as a similar lag was seen on a beach treated with fertilizer briquettes (data not shown). If decay rates are compared following the lag (between the 6/25 and 8/26 sampling dates), the fertilizer treatment produced a decay rate approximately 2 times that seen on the control beach. Therefore, based on the assumptions described above regarding the meaning of the ratio changes, biodegradation was

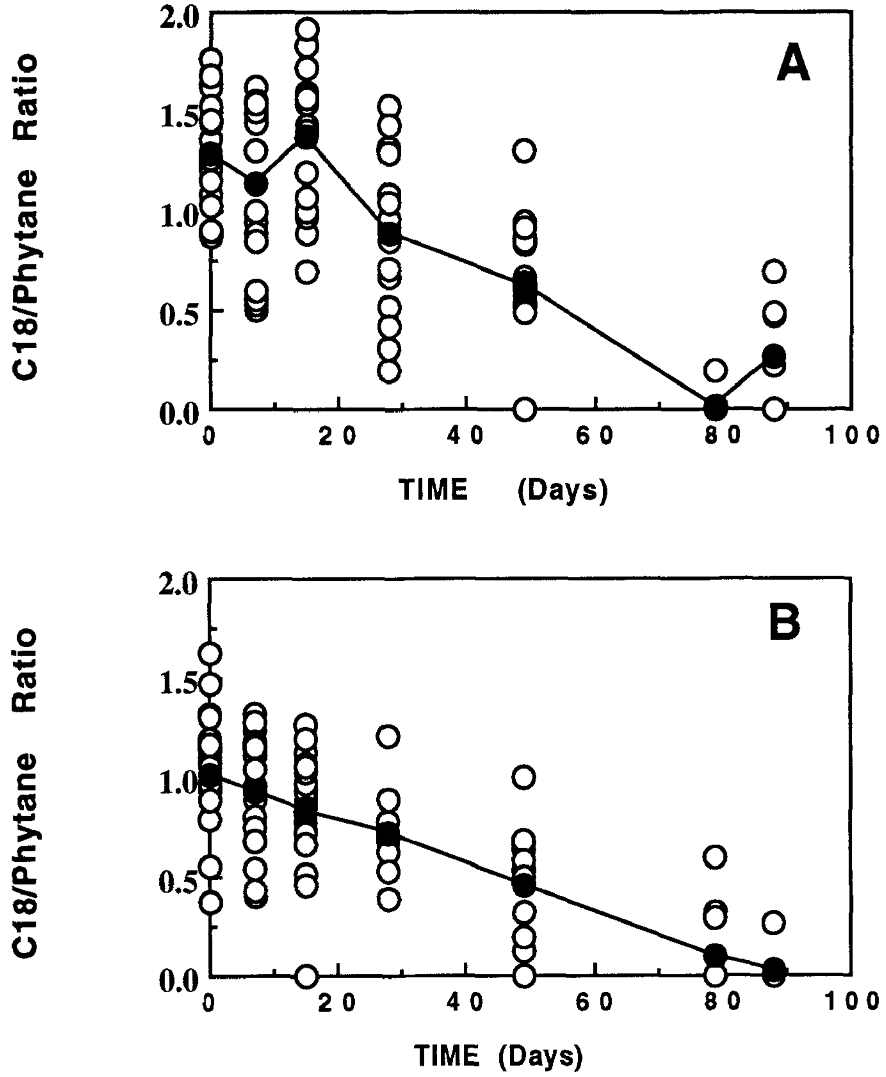

Fig. 4. Changes in the n-C18/phytane ratio through time in oil extracted from cobblestone samples from Inipol fertilizer-treated (A) and untreated (B) beach plots in Snug Harbor. Shaded symbols are means. Fertilizer was applied immediately following the day 0 sampling (6/8/89).

occurring on both beaches, but appeared to be enhanced by the application of the oleophilic fertilizer. This corresponds with the visual observations discussed above. The sustained nature of this enhancement is interesting given that the release of nutrients from the fertilizer probably occurred over a considerably shorter time period.

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. 4 (many samples showed evidence of extensive biodegradation while others showed very little) was a function of the highly heterogenous 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, thereby diluting 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 (data not shown). The absence of a change in the n-C18/phytane ratios through time for the subsurface mixed sand and gravel samples suggested that oil biodegradation was not occurring. Examination of phytane itself in the mixed sand and gravel under the cobble on the Inipol-treated beach showed that its loss was as fast as that for n-C18 alkane. Biodegradation was either 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 (Prinik et al. 1977; Mueller et al. 1992) and we have readily 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 a internal biological marker 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. Why phytane degradation was possibly much faster in mixed sand and gravel is not clear but it may be related to the greater surface area available. Again, if degraded oil was not removed physically as it was on the beach surface, continued degradation of the degraded oil would occur; bacteria on the beach surface, however, would continually be exposed to fresh undegraded oil as the degraded oil was removed. This could also, explain the visual observation of more oil loss on the beach surface.

Alternatively, results from the mixed sand and gravel samples under the cobble could suggest that Inipol was acting in a chemical manner (surfactant effect) to remove aliphatic and branched hydrocarbons simultaneously. However, n-C18 and phytane disappeared in essentially the same manner in mixed sand and gravel samples from the untreated control beach. Since there was no possibility of a chemical effect on the control plot, the conclusion is that phytane removal was primarily due to biodegradation, although this cannot be proven.

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 presented above, biodegradation of the aliphatic fraction was not sufficient in itself to establish the overall effectiveness of bioremediation. Thus, another approach for examining biodegradation effects was to perform selective mass ion spectrometry following gas chromatographic analysis. A variety of aromatic hydrocarbons, that are perhaps more difficult to degrade than the aliphatic hydrocarbons, can be examined with this method (Kennicutt 1988; Rowland 1986). This was important not only because it tracks another degradable fraction of the oil, but because certain polycyclic aromatic hydrocarbons (PAHs) are known to be potential pro-carcinogens 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 none the less an additional factor to be considered in effectiveness assessments.

Furthermore, as the low solubility of the PAHs makes them to difficult to degrade, they can be used as an indicator of the extent 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 3. 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 multi-ring 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. None the less, hopane provides a consistent standard to normalize the concentrations of aromatic hydrocarbons.

From Table 3, the relative differences 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. Despite the limited number of analyses, samples that showed low n-C18/phytane ratios also showed large changes in many of the aromatic and heterocyclic hydrocarbons. Of the 58 PAHs monitored, only a few remained in samples with a low ratio. There are insufficient analyses at this time to demonstrate a further relationship between the amount of ratio reduction and the amount of aromatic hydrocarbons remaining. One cannot explicitly state that disappearance of the aromatic and heterocyclic hydrocarbons was due to biodegradation but the suggestion is strong. Many of the higher molecular weight PAHs are unlikely to be affected by chemical or physical processes because of their low solubility and volatility. Biodegradation may be the only mechanism that could explain their disappearance. In addition, these samples were taken from the beach approximately 50 days after the application of the Inipol fertilizer and any chemical effect from the fertilizer that could cause these changes in composition was equally unlikely.

Samples with reduced n-C18/phytane ratios also showed increased concentrations (percent of total oil residue weight) of the hexane insoluble (but methylene chloride soluble) fraction. Chemically, this fraction should consist primarily of asphaltene hydrocarbons (Speight et al. 1984) which are quite resistent to biodegradation. However, polar materials originating from the partial oxidation (most likely by bacteria) of high molecular weight hydrocarbons would also accumulate in this fraction. Samples with increased percentages of asphaltene material relative to Prudhoe Bay crude suggest that considerable removal of other components in the oil had occurred, possibly because of biodegrada-

Table 3. Relative concentrations of aromatic, hererocyclic and cyclic hydrocarbons normalized to hopane in oil from cobble surfaces in Snug Harbor.

	Oleophilic fertilizer treatment		
	Purdhoe Bay Crude[a]	Day 0	Day 50[b]
n-C18	32.28	22.05	0.30 (0.23)
Phytane	15.2	15.62	1.7 (1.16)
n-C18/Phytane ratio	2.08	1.41	0.46 (0.30)
C3/Naphthalenes	11.92	2.51	0.00
C3/Fluorenes	2.67	2.24	0.01
C3/Phenanthrenes	3.75	3.84	1.11 (0.39)
C2/Pyrenes	1.14	0.92	0.27 (0.16)
C3/Dibenzothlophene	3.97	4.14	1.28 (0.47)
C2/Chrysenes	1.15	0.98	0.62 (0.25)
Benzo (e) pyrene	0.18	0.15	0.10 (0.05)
Total PAHs[c]	58	29	3.8 (0.8)
% Hexane insoluble weight	6%	11%	36.2% (21.5)

[a] Artificially weathered to simulated oil found on Prince William Sound Beaches (Lindstrom et al. 1991).
[b] Mean (standard deviation) N = 5; randomly selected from the 8/26/89; all samples 50 mg of extracted oil residue per ml.
[c] Number of PAH peaks monitored in mass spectral analysis.

tion. However, this percentage increase should also occur with hopane, as it is also a conserved internal biological marker (Kennicut 1988; Lindstrom et al. 1991). Results showed hopane only increased slightly, whereas the hexane insoluble fraction increased (percent of total oil residue weight) almost six-fold. The lack of correspondence between hopane and the hexane insoluble fraction is difficult to explain in chemical terms. However, it does make sense biologically if we assume that biodegradation products are accumulating in the hexane insoluble fraction through time. Controlled laboratory experiments involving the generation of extensively biodegraded oil, support this interpretation (P. Chapman, pers. comm.). If this is the case in the field, clearly oil residues remaining on the beach experienced considerable biodegradation.

Relationship of compositional change to residue weight change

A relationship was then established between compositional changes and the loss of oil residues from beach material. Due to limitations of the analytical procedures routinely used to measure hydrocarbon composition, less than 10% of the oil by weight is represented, as was discussed above. Chemically analyzing the remaining 90% of the oil to improve on measures of effectiveness was not practical because of cost and analytical complexity. Thus, some bulk measurement of oil degradation was needed. Since bioremediation is designed to ultimately degrade as much oil as possible from contaminated beaches, monitoring changes in total oil residues seemed to provide the best measure of biodegradation extent. However, this is only reasonable if some correlation can be established between changes in oil residue weight and changes in hydrocarbon compositions, since it is the latter that can be linked directly to biodegradation.

Figure 5 shows a plot of the changes in residue weight against changes in the n-C18/phytane ratio. 'Change' in this case is expressed as the percent reduction of the median values (from 21 field samples at each sampling time) relative to the $t = 0$ sampling for each parameter. Median values were used in this case because of their independence from out-lying data points and because they are probably a good representative of the samples taken on a particular sampling date. The data points reflect the sampling times with the progression of time going from right to left in the figure. A positive correlation was apparent. The differences in slopes suggests that for Inipol-treated plots biodegradation (i.e., change in the n-C18/phytane ratio) were accompanied by a greater removal of oil residue. The results strongly suggest that reductions in the ratio consistently accompanied reductions in oil residue weight or vice versa. This was unexpected since the n-C18 alkane, being one of the most rapidly degraded hydrocarbons, could easily be reduced to the point of rendering the ratio technique ineffective, prior to extensive change in the oil residue weight. However, this was apparently not the case. The results can possibly be explained by assuming that oil biodegradation worked at the surface of an oil layer covering the cobble and that degraded oil residues continuously sloughed from this layer, leaving behind largely undegraded oil. When oil was extracted from the cobble surface during sampling, a mixture of degraded and undegraded oil was obtained, with the undegraded oil providing most of the measured amount of n-C18 alkane (and phytane). With time, the undegraded oil became less and less of the total oil residue recovered. Thus, in a sense, the degradation of normal and branched alkanes was internally compensated as a function of the rate at which bacteria at the oil–water interface were exposed to undegraded oil.

Based on these results, reductions in oil residue weight can be used as a direct measure of biodegradation and the effect of fertilizer addition on biodegradation rates assessed. The decrease in oil residue weight from cobble surfaces on Inipol-treated and untreated control beaches is shown in Fig. 6. Assuming that decay rates for the oil residues were first order, slopes and half lives of the oil can be calculated (Table 4). Application of oleophilic fertilizer caused an apparent greater than two-fold increase in the loss rate of oil residues. The effect, was not unfortunately, statistically significant (p = 0.05) due to the large variability in the data. Other statistical treatments are currently being examined in an attempt to significantly verify the effect.

We, therefore, 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 nutrients seemed to produce a sustained enhancement effect. 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 could remove (assuming linearity) approximately 2.5 times more oil from the cobble surface than on the untreated control beach. Coincidentally, respiratory activities by oil degrading microbial communities on fertilized beaches has been shown to increase an average of 3-fold (Lindstrom et al. 1991). Thus with an initial concentration of 1.0 mg of oil per gram of beach material (cobble surface), bioremediation 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 may be related, as we discussed above, to their being partially trapped in the beach matrix. On the other hand, 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 centimeters 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 on the day 2 sampling (Table 5). 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

Table 4. 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[a] 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

[a] Only the INIPOL rate is significantly different from zero (p = 0.05); slopes were significantly not different from each other (p = 0.05).

Table 5. 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[a]			
	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

[a] Blocks were 5 m long and 4 m wide running end-to-end parallel to the water line and covering 3 parallel zones, each 4 m wide running side-to-side up the beach. Blocks 2, 4, and 6 in each zone were not sampled.

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 would also result in increases in the number or activity (growth rate) of oil-degrading bacteria. To determine if this was the case, beach samples (mixed sand and gravel) were analyzed using an MPN 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 three months sampling period (data not shown). However, as indicated above, the concentrations of oil degraders was 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).

Mechanism of oleophilic fertilizer action

Despite the success of enhancing oil biodegradation rates through the application of fertilizers, the mechanism of action of the oleophilic fertilizer was still not clear. Was it enhancing oil biodegradation by the supply of nitrogen and phosphorus or by some other mechanism? Or was it causing removal of the oil by some chemically-enhanced process?

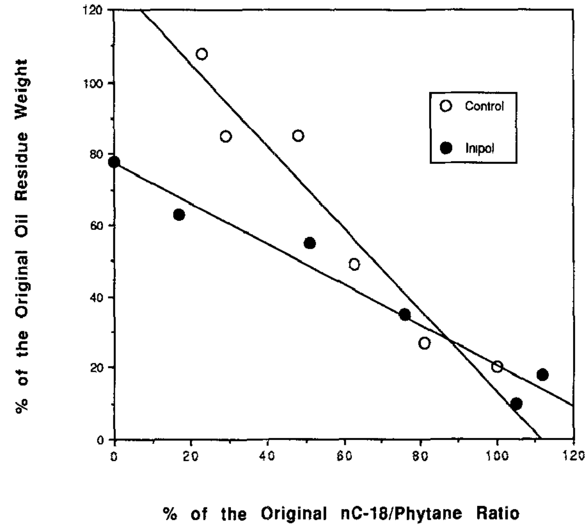

Fig. 5. Relationship of changes in oil residue weight to changes in the n-C18/phytane ratio (expressed as % reduction of the median at each sampling interval) from oil samples taken from cobble surfaces for both Inipol fertilizer-treated and untreated beach plots in Snug Harbor. Data points at the lower left corner of the graph represent samples taken later in the sampling period.

To answer these questions, a series of laboratory studies was conducted. All experiments used oiled beach material from Prince William Sound and the test system consisted of the biometer shake flasks described above. Incubations were carried out at 15 °C using a 12-hour tidal cycle: beach material was incubated without overlying sea water (low tide) for 12 hours then covered with seawater (high tide) every 12 hours and gently shaken at 75 rpm. Fertilizers were applied either to the overlying water (soluble nutrients) or directly to the oiled beach material at 'low tide' (Inipol EAP 22). Biodegradation activity was measured as either total CO_2 production or as ^{14}C-CO_2 production from radiolabelled phenanthrene. Carbon dioxide was trapped in dilute NaOH (0.1 N) contained in the flask side arm (Fig. 1). Phenanthrene, which was added by dissolving in the tidal water, completely sorbed to the oiled beach material and consequently its min-

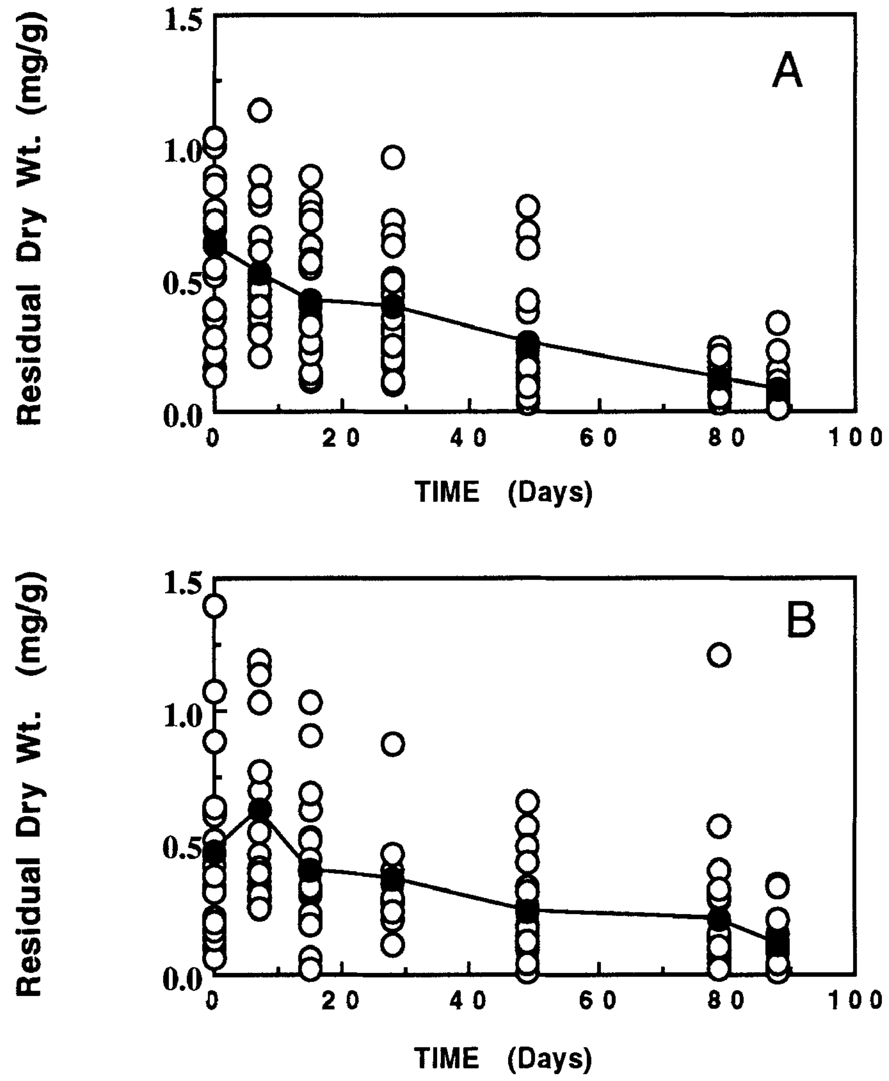

Fig. 6. Changes in oil residue weight through time extracted from cobble stone samples from Inipol fertilizer-treated (A) and untreated (B) beach plots in Snug Harbor. Shaded symbols are means. Fertilizer was applied immediately following the day 0 sampling (6/8/83).

eralization was probably catalyzed by the microbial communities on the surface of the oil.

To determine if Inipol was enhancing oil biodegradation by nutrient supply alone, flasks were set up to compare the effect of soluble nutrients with Inipol. Figure 7 shows that mineralization of the oil (oil was the dominant carbon source by far so we assumed that most of the CO_2 was from the oil and not other organic material) was enhanced by both the addition of soluble nutrients and Inipol. Where the total concentration of nitrogen was the same for the two different fertilizers (Inipol and low level soluble nutrients), mineralization enhancement was approximately the same. These results argue that the other organic materials in the Inipol have no effect on the mineralization and it was the concentration of nitrogen supplied that was important. Unfortunately, experiments of greater duration were not performed and thus the long term effect of Inipol carbon cannot be assessed. Similar laboratory experiments have shown that phosphorus stimulated mineralization only to a minor extent. Some of the CO_2 production in these experiments was undoubtedly due to the mineralization

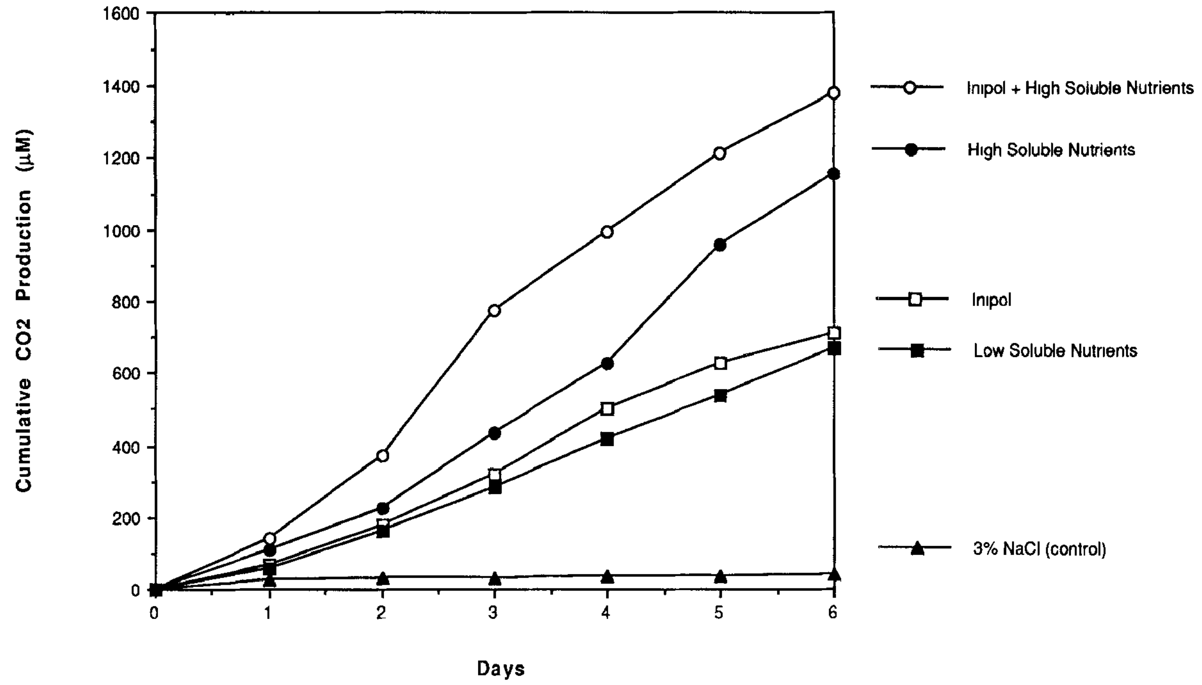

Fig. 7. Effect of Inipol fertilizer and soluble inorganic nutrients on the mineralization of oil in mixed sand and gravel samples from Prince William Sound.

of organic materials in the Inipol fertilizer but Inipol carbon accounted for < 1% of the total oil carbon in the flask.

Additionally, only the replacement water ('high tide') on the first day was supplemented with nutrients; all subsequent replacements had no added nutrients. Thus, the mineralization results reflected the effect of a single pulse exposure to the nutrients at high concentrations. If we assume that much of the Inipol nitrogen was released during the first exposure to water, then it too could represent a single pulse of nitrogen at high concentrations.

Where Inipol was combined with a high level of soluble nutrients (Fig. 7), more mineralization occurred than with the high level nutrients alone. Again, this extra mineralization can be accounted for by the additional nitrogen provided in the Inipol fertilizer.Thus the ability of the oleophilic fertilizer to 'hold' nitrogen close to the bacterial communities involved in oil biodegradation cannot be directly verified with these experiments, since a simple single exposure to nitrogen in any form may have been enough to enhance oil biodegradation to an equal degree.

An additional set of experiments was conducted to ascertain the importance of the individual chemical components in Inipol. Equivalent samples of oiled beach material where exposed to oleic acid and tri(laureth-4) phosphate at concentrations approximately equal to that from an Inipol application, and the flasks incubated in the presence of soluble nutrients in the 'tidal water'. There was no effect due to these organic materials, as the amount of mineralization was no greater than that with the soluble nutrients alone (Fig. 8). Soybean oil was also checked as it has high concentrations of oleic acid and nitrogen containing organic compounds (e.g., amino acids); i.e., like a natural oleophilic fertilizer. Unfortunately, soybean oil was essentially ineffective. Despite the conceptual appeal of soybean oil, either the nitrogen content was too low or it was not released as fast as nitrogen from the urea in Inipol.

Inipol, then, appeared to serve as an effective nutrient source for oil-degrading microbial com-

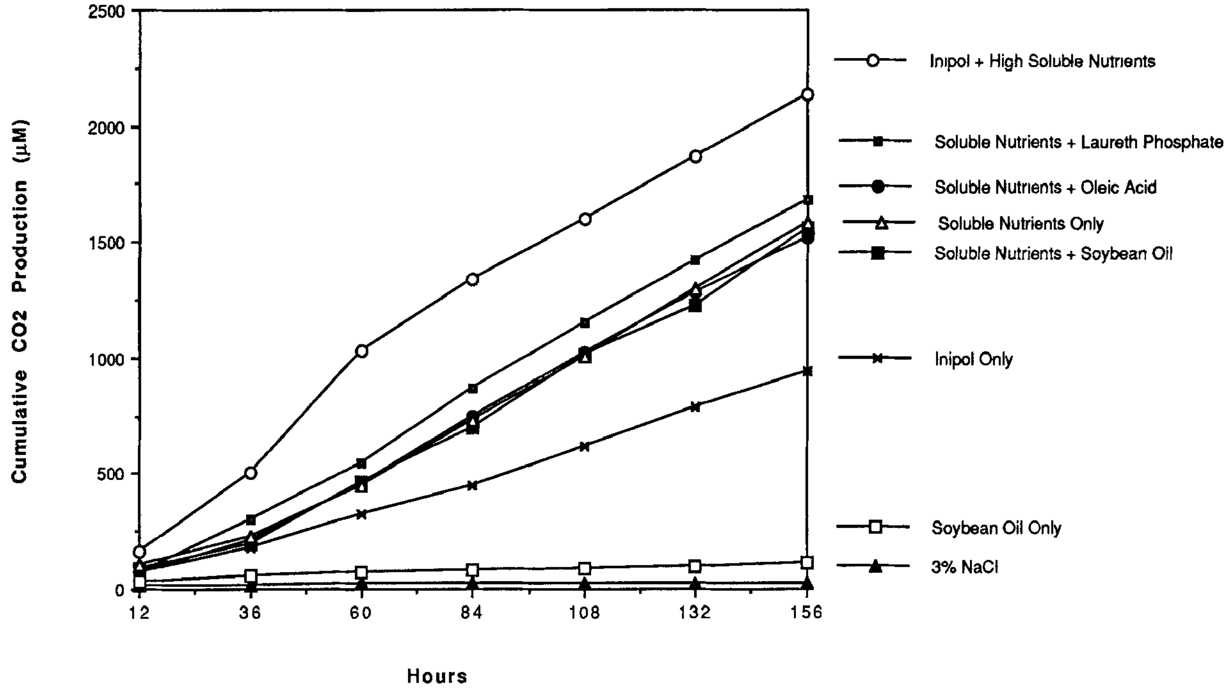

Fig. 8. Effect of Inipol fertilizer, soluble inorganic nutrients and other organic materials on the mineralization of oil in mixed sand and gravel samples from Prince William Sound.

munities but the contribution of its oleophilic nature for the bioremediation of contaminated beach material from Prince William Sound is still unclear.

Summary and conclusions

The results from our field demonstration of oil spill bioremediation in Prince William Sound has shown that the use of the oleophilic fertilizer, Inipol EAP 22, served as an effective nutrient source for oil-degrading microbial communities. It appeared to enhance oil biodegradation, as measured by both changes in oil composition and oil residue weights, by as much as two-fold compared to the untreated controls although this effect could not be statistically verified due to high variability in field samples. The Exxon Corporation and regulatory authorities believed this was enough of a response to merit incorporation of bioremediation on a large scale into the remedial action cleanup plan for oil contaminated beaches in Prince William Sound. Our studies support the belief that the visual observation of oil removal from the beaches 8–10 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 potentially made these beaches safer for local wildlife (less tendency for fur and feathers to become oiled) and minimized the movement of undegraded oil from the beaches into the water column. These changes occurred in a shorter period of time then 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, has been emphasized as a key element in this success. In addition, the 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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