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OVERVIEW OF EXXON CORPORATION'S BIOREMEDIATION PROGRAMS WITH EMPHASIS ON THE ALASKA SPILL

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ABSTRACT

Following the Valdez spill in Alaska in 1989, Exxon scientists worked closely with the U. S. Environmental Protection Agency (EPA) and the State of Alaska, to conduct the most extensive bioremediation study on oiled shoreline ever carried out. Over $10 million was spent conducting this research. The process involved applying fertilizers on intertidal shorelines to stimulate the growth of indigenous oil-degrading microbes and accelerate the rate of natural degradation of the oil. In analyzing the data from this program, Exxon developed unique approaches, focusing on specific changes in the oil chemistry to verify the degradation rate increase provided by the added nutrient. Such a sophisticated approach was required because of the problems inherent with reliance on the measurement of total petroleum hydrocarbon in the sediment to define the degradation rate. Results show that the rate of oil biodegradation on fertilized beaches was from three to more than five times faster than on unfertilized beaches.

This paper summarizes the bioremediation research conducted, and the extensive information obtained. The experience gained by Exxon, including pioneering work on internal markers, applies directly to bioremediation studies in other environments. Some of this experience is being extended to the remediation of inland contaminated sites and there is a major effort now underway to identify low-cost bioremediation techniques for in-situ restoration of such sites.

OVERVIEW OF THE ALASKA CLEANUP

On March 24, 1989, the tanker "*Exxon Valdez*" ran aground on Bligh Reef, releasing about 41,000 tons (11 million gallons) of Alaskan North Slope crude oil into Prince William Sound, Alaska. A portion of this oil floated ashore. Altogether, some 2100 km (1300 miles), or 15% of the total shoreline in Prince William Sound and the Gulf of Alaska, was oiled to some degree (Owens 1991).

Oiling was heaviest on islands directly in the path of the slick (southwest of the spill site). It was essential that the heaviest concentrations of oil be removed as soon as possible to minimize contact with wildlife and fishery resources and to prevent the oil from being remobilized with the risk of impacting other shorelines. The massive cleanup force, required because of the extensive shoreline affected, peaked in 1989 at over 11,000 workers, 1400 boats, and 84 aircraft. By mid-September 1989, all oiled shoreline approved for treatment had been treated by one or more processes, including 120 km (74 miles) treated by bioremediation.

By 1990, shoreline oiling had decreased dramatically as a result of the 1989 cleanup activities and natural cleansing by winter storms (Jahns et al. 1991). In 1990, bioremediation was the primary tool used both on a stand-alone basis and in combination with mechanical cleanup techniques, such as storm berm relocation, tilling, and manual pickup. By 1991, oiling was further significantly reduced, and the level of bioremediation effort needed was only about one-seventh as much as in 1990.

A joint federal and state shoreline survey conducted in May-June 1992 confirmed that essentially all oil had been removed from the shoreline. On June 12, 1992, the US Coast Guard (USCG) and the State of Alaska officially declared the cleanup concluded, indicating that further cleanup activities would not provide a net environmental benefit (NEB), i.e. subsequent cleanup operations would do more harm than good.

This paper summarizes involvement by research scientists at Exxon and EPA in the bioremediation phase of the cleanup.

SELECTION AND ROLE OF BIOREMEDIATION

Within two weeks of the Valdez grounding, Exxon formed a special task force made up of scientists and engineers to provide technical support for the field cleanup teams. Exxon has had an ongoing oil spill response R&D program for 20 years -- the largest in the oil industry -- and was able to draw on extensive experience in assembling the support teams. Early in the cleanup, Exxon and EPA scientists jointly proposed the possibility of using bioremediation as one approach for quickly treating the impacted shoreline. The first step was to confirm that microorganisms with the ability to degrade oil were abundant in Alaska waters. Hydrocarbon-degrading microbes have been studied extensively, and they have been found in all marine environments sampled (Robertson et al. 1973, Mulkins-Phillips and Stewart 1974, Bunch and Harland 1976, Roubal and Atlas 1978, Gunkel et al. 1980). In general, researchers have found that in marine areas far from any source of petroleum hydrocarbons the numbers of oil-degrading organisms are lower than near sites of petroleum discharge. Hydrocarbon-degraders normally make up less than 0.1% of the total microbial population in areas removed from unusual hydrocarbon sources such as natural submarine seeps or municipal/industrial discharge sites (Atlas 1981), but increase their fraction of the total microbe population to 10% or greater near sources of oil discharge (Mulkins-Phillips and Stewart 1974).

Water samples collected just offshore of an oiled beach in April 1989 revealed that there were indeed a broad range of oil-degrading microbes in Prince Willliam Sound, and that they had already responded to the presence of oil by increasing in number to between 1-10% of the total microbial population. Their initial presence was later attributed to the hydrocarbon compounds falling from the numerous evergreen trees in the area. This was very encouraging and indicated that bioremediation would have a high chance for success. This was later verified in the field.

The next step was to consider the chance of success based on prior experience. A key issue was how to retain fertilizer in an intertidal environment long enough to work before being washed out. Prior to the *Exxon Valdez* accident, bioremediation of marine oil spills had only been studied in laboratory tests and a limited number of field trials (see, for instance, Lee and Levy 1986; 1989). Interest in using bioremediation to clean marine oil spills was stimulated following the *Amoco Cadiz* spill in 1978, when it became clear that biodegradation of hydrocarbons had played a major role in natural oil removal (Gundlach et al. 1983). There was evidence that fertilizers in water from agricultural runoff had accelerated biodegradation.

Recognizing the need for a liquid fertilizer that would allow slow release of nitrogen and phosphorus and remain with the oil to be degraded, Elf Aquitaine developed Inipol EAP22 (Ladousse and Tramier 1991), which is an oil-external microemulsion that contains nitrogen and phosphorus nutrients. Because it contains an external oil phase, it readily sticks to oil residues and is therefore called an "oleophilic" (oil loving) fertilizer.

This fertilizer was first tested against a water-soluble agricultural fertilizer in an experimental spill in Norway (Sendstad et al. 1984; Halmo 1985). A three fold increase in biodegradation of alkanes was observed for Statfjord oil fertilized with either Inipol or agricultural fertilizer relative to oil from unfertilized areas, but with no statistical difference in rate between the two fertilizers.

Inipol EAP22 was used following an actual spill in November 1985 of about 88,000 liters of marine gas oil on the shoreline in Ny Alesund, Spitsbergen (Sveum 1987; Sveum and Ladousse 1989). The fertilizer was applied the next summer, and extensive monitoring of hydrocarbon levels was conducted. The increase in biodegradation rate of treated oil was reported to be about six to nine times higher than for untreated oil.

Overall, available experience suggested that addition of fertilizer could have significant potential to help eliminate oil stranded in intertidal zones in Alaska. However, because prior tests were viewed as limited and with results that might be site specific, success of field bioremediation following the *Exxon Valdez* spill could not be assured without field testing. There were also questions about the safety of bioremediation application.

1989 FIELD AND LAB TESTS

To test the effectiveness and confirm that bioremediation would be safe, the U. S. Environmental Protection Agency (EPA) and Exxon executed a joint research agreement and undertook extensive field and laboratory test programs in the summer of 1989. Based on advice from international scientific experts provided at a conference hosted by EPA, the emphasis was on simply adding fertilizers to the beaches to supplement naturally available nitrogen and phosphorus. No trace metals or other micronutrients were included and no microorganisms or enzymes were added. The bacteria involved were strictly native bacteria already present on the Alaska shoreline.

The EPA effort focused on field activities: conducting initial pilot demonstration tests of bioremediation, evaluating various fertilizers for effectiveness, and developing treating procedures for large-scale applications by Exxon if the tests were successful. Exxon's effort, in addition to logistical and operations support, was directed at conducting laboratory screening studies to support the EPA field demonstration. Exxon's laboratory testing program was designed to confirm that microbes from Prince William Sound would degrade Prudhoe Bay crude oil and to determine how much faster biodegradation might proceed if fertilizers were added to shorelines.

The fertilizers selected for field testing in 1989 had to meet practical criteria imposed by the short response time and be commercially available in large quantities, amenable to practical transportation and application strategies, and able to deliver nitrogen and phosphorus effectively. Designed to provide nitrogen and phosphorus nutrients to the indigenous bacteria over a reasonably sustained period of time, three different application strategies were considered:

- Oleophilic liquid fertilizer,
- Slow-release solid fertilizer, and
- Aqueous fertilizer solution

Oleophilic liquid fertilizers are designed to adhere to oil and to shoreline substrate (e.g., rocks, gravel), providing a reasonably uniform surface coating near the oil to be degraded. Nutrients are slowly released to the water near the oil-water interface where they can be utilized by microbes.

Slow-release solid fertilizers, developed for agricultural use, utilize formulations designed to dissolve slowly or break down on continued or intermittent contact with water and release various forms of nitrogen and phosphorus. Several candidates were available for testing.

Aqueous fertilizer solutions contain soluble forms of nitrogen and phosphorus and are applied by spraying through water sprinklers. The basis for this application was that solutions of known nutrient concentrations could penetrate sediment and provide rapid availability of nutrients with uniform coverage. The aqueous fertilizer solution was not considered a candidate for large-scale application in 1989 because there was insufficient lead time for developing a practical delivery system. However, this type of fertilizer was included in the research program for possible use beyond 1989.

After several different products and application strategies were tested, the oleophilic fertilizer Inipol EAP22 and the slow-release granular solid Customblen were selected for large-scale use. Table 1 provides the components contained in each.

Inipol EAP22, manufactured by a subsidiary of Elf Aquitaine (Elf Aquitaine 1982), and hereinafter referred to simply as Inipol, was the only oleophilic fertilizer available for large-scale application. It is a honey-colored liquid microemulsion with an external phase of oleic acid, the principal fatty acid of olive oil. The internal phase consists of micron-sized droplets of water containing urea, the nitrogen source. These droplets are stabilized within the oil by tri(laureth-4) phosphate, frequently used in cosmetics, which also provides the phosphorus source. A fourth component, 2-butoxyethanol, serves to further stabilize the microemulsion and reduce its viscosity. For early testing, Inipol was sprayed on the oiled sediment by back-pack sprayers, but airless paint sprayers were adapted later for large-scale applications.

Customblen granular fertilizer, manufactured by Grace Sierra Chemicals, was developed as a high-quality lawn and agricultural fertilizer with slow-release properties.

Exxon's early screening experiments showed that indigenous bacteria from Prince William Sound were capable of degrading many hydrocarbon components in the residue oil. These laboratory screening tests confirmed that the addition of fertilizer to the beaches had a high probability of increasing the rate of oil degradation by indigenous microorganisms if sufficient nutrient concentrations could be maintained within the sediment. The ability to achieve such nutrient concentrations in the dynamic beach environment could be answered only by field tests.

The EPA initiated field work in early May 1989 to identify appropriate sites for demonstration tests. Snug Harbor and Passage Cove on Knight Island were selected since the oiling and geomorphological conditions appeared to be well suited for bioremediation (i.e. no pooled oil, permeable substrate, etc.). These beaches consisted mainly of cobblestones overlying a base of mixed sand and gravel. Both sites contained oily residue on the cobble surfaces and oil mixed into the sand and gravel under the cobblestones.

Eight to ten days after the application of Inipol at Snug Harbor, a striking reduction of oiling was visible in the treated cobble plot. Close examination of the beach showed that much of the oil on the cobble surfaces was gone, but oil still remained in the gravel and sand under the cobble covering. Also, there appeared to be a reduction in the oiling on the Inipol-treated gravel beach after two to three weeks, but the effect was not as striking. Substantial changes in oil composition occurred on all test plots over the three months of monitoring.

Oil degradation was clearly occurring on both fertilized and control plots, but differences between fertilized and control plots were not statistically significant. Oil degradation was determined by the absolute loss of alkanes, and by a decrease in the nC18-phytane ratio. This later ratio has been widely used to follow degradation, since microbes degrade the linear alkane (nC18) more readily than the branched phytane, this ratio decreases as biodegradation proceeds. It was subsequently shown that the indigenous microbes of Prince William Sound were degrading phytane far faster than expected, so that changes in the nC18-phytane ratio substantially underestimated the rate of biodegradation.

Results from ecological monitoring in these tests were very encouraging. Nutrient concentrations did not increase in waters adjacent to the treated shoreline. Chlorophyll analyses of phytoplankton samples showed fertilizer applications had no measurable impact on algal growth, and all chlorophyll values were within the expected range for Prince William Sound. Mussels caged just offshore of the test plots showed no detectable PAHs, suggesting that the fertilizers did not increase the input of petroleum compounds into the water column. Furthermore, no oil slicks were observed along the shoreline following fertilizer application.

Considering all available data, including microbiology studies, decreases in sediment oil loading (Figure 1), changes in oil chemistry, and visual improvement of shorelines, the EPA concluded that bioremediation enhanced oil removal. However, EPA was not able to provide statistically significant quantitative measures of biodegradation due to the highly heterogeneous nature of the oil and sediment. This issue was addressed in subsequent laboratory test programs and in the 1990 field testing program. Nevertheless, the data obtained in 1989 showed that bioremediation was safe and likely to be effective, and approval was granted by state and federal authorities for widespread application. Between August 1 and September 15, 1989, over 120 km (74 miles) of shoreline in Prince William Sound and the Gulf of Alaska were treated primarily with Inipol EAP-22 and some Customblen slow release granular fertilizer. Large-scale applications of both these fertilizers continued during the summers of 1990 and 1991, and small quantities of Customblen were used on the few isolated patches of oil remaining in the summer of 1992.

1989-1990 LABORATORY STUDIES

The focus of laboratory research late in 1989 and early 1990 was on the potential for subsurface bioremediation, a better understanding of the mechanism by which Inipol works, and on toxicology issues. It became clear that the dramatic cleansing seen with Inipol EAP22 was indeed a biological process, and that Inipol EAP22 had no "rock-washing" properties. Furthermore, biodegradation was proportional to the amount of available nitrogen in the concentration range used in the bioremediation applications, and almost all classes of oil compounds (paraffins, naphthenes, 1-,2-, and 3-ring aromatics and polar compounds) were subject to degradation, with the exception of the asphaltenes which made up less than 2% of the original oil (Chianelli et al, 1991). Beach simulators were constructed to test how effective fertilizer applications to the surface of the beach were in providing nutrients to subsurface bacteria. The results of these pilot tests were very encouraging.

1990 MONITORING PROGRAM

Based on results from the Exxon laboratory tests that demonstrated likely success in subsurface bioremediation and on additional toxicity tests by the EPA that indicated minimal risk to shoreline biota, approval was granted in April 1990 for continued large-scale application of bioremediation in 1990 to treat remaining surface and subsurface oil. However, the State of Alaska approved bioremediation contingent on Exxon's funding and conducting a research monitoring program jointly with the EPA and the State of Alaska. That monitoring program, conducted on three test beaches on Knight Island, produced the most extensive field data on bioremediation available to date. The statistical analyses of those data ultimately provided quantitative proof of effectiveness of bioremediation, and a technically sound explanation of why results were better on some beaches than others. The program, initiated in late May 1990, was designed to provide data within six weeks to assist in decisions on re-applications of bioremediation (Prince et al. 1990, Prince 1992, Prince et al. 1993). The program was extended into September to more fully characterize efficacy and environmental effects of the treatments.

To assess efficacy, the program focused on:

- Ability of nutrients to penetrate and remain in pore waters within the sediment,
- Stimulation of the ability of the microbes to degrade hydrocarbons ("mineralization potential"),
- Changes in number of microbes in response to fertilizer addition, and
- Changes in amount and composition of oil residue in the sediment.

The main strategy of the tests was to compare changes in oil characteristics on a fertilized beach with changes in oil on an adjacent, untreated control. Key criteria for site selection were the size and similarities in sediment structure and oiling of the segment. Each beach needed to be sufficiently large to permit dividing it into a fertilized area and an untreated control area having similar properties.

Test beach KN-135 (Figure 2) was a low-energy beach located in Bay of Isles that contained both surface and subsurface oil. KN-211 was a high-energy beach facing the original spill that contained primarily subsurface oil. The third site, KN-132, was a low-energy beach in Herring Bay that contained only surface oil. All three beaches were more heavily oiled than the majority of shorelines that were bioremediated in 1990, and, except for a small section of KN-211, none had been bioremediated in 1989.

During the 100 days of testing, samples of interstitial water were collected and analyzed periodically for nutrient levels, dissolved oxygen, salinity, pH and temperature. Sediment samples were also collected from both the surface and subsurface to determine residue oil weight and composition and microbial numbers and activity. Toxicology tests were conducted using water samples collected from the beaches. Waters were tested for chlorophyll concentration to determine if algal stimulation was occurring.

Detailed summaries of the Monitoring Program findings have been provided in Prince et al. 1990, Lindstrom, et al. 1991, and Bragg, et al. 1992 and are briefly summarized here:

- There were no adverse ecological effects.

- Significant reductions in oiling on beaches were visually apparent within one month of fertilizer addition even though the oil had been in place for 14 months prior to testing.

- Rates of degradation were substantially different on the sites tested. This relates to the degree of degradation which had already occurred prior to the test. In particular, the oil on KN-132 was substantially more degraded than that on KN-135 so that it was more difficult to stimulate degradation on KN-132.

- Response to the addition of fertilizer varied on the three beaches. For example, there was a significant release of nitrogen into the interstitial water on KN-135 and KN-132, however, there was virtually no nitrogen detected in KN-211 until a second application at day 44. There is evidence that Customblen washed out of KN-211 after the first application, complicating the data interpretation.

- There was no substantial increase in phosphate in the interstitial water.

- Oxygen consumption in interstitial water peaked about two weeks after the first application of fertilizer, declined back to background levels after one month, and then peaked again following re-application (Figure 3), consistent with nitrogen concentration. However, there was always adequate oxygen present.

- More organisms were generally present on the fertilized beaches, especially in the subsurface.

- Laboratory mineralization experiments exposing organisms from the test beaches to radioactive tracer hydrocarbons showed an activity enhancement of four times for fertilized surface samples and up to ten times for subsurface samples.

- Initial attempts to analyze the sediment samples for residue oil to show statistically significant differences with and without fertilizer were frustrated by the extreme heterogeneity of oil loadings from sample to sample.

DATA INTERPRETATION BREAKTHROUGH

The problems Exxon and EPA encountered in providing statistically significant measures of biodegradation based on sediment oil analysis alone proved to be a major obstacle in obtaining widespread acceptance in the scientific community that fertilizer addition to the beaches had indeed achieved the rate enhancement being claimed by the investigators. This also proved to be a problem in another bioremediation application carried out by Exxon off Staten Island, New York in 1990 (Madden et al. 1991). Though physical evidence was compelling, statistical verification was not possible. In a dynamic beach environment, gravimetrically measuring change over time in the mass of oil caused only by biodegradation is virtually impossible because other physical processes are simultaneously causing loss or gain of oil. As sediments are exposed to waves and tidal currents, oil residues can be remobilized, leading to localized changes in the amount and specific location of oil (Owens 1991; Jahns et al. 1991). Physical removal of oil by natural processes can cause a reduction in oil loading without a net change in oil composition. Also, oil loading can increase locally because of remobilization from adjacent sediments even as the oil is being biodegraded, so gravimetric measures alone provide a misleading picture of bioremediation effectiveness.

The other complication, cited earlier, was that the approach used in earlier spills to monitor rate of biodegradation by tracking changes in the ratio of normal to branched alkanes (n C18/phytane) was confounded by the adeptness of Alaskan bacteria in consuming the phytane.

To overcome these barriers Exxon began a search for alternative markers in late 1989 as part of its extensive Winter Laboratory Program. In collaboration with Battelle Ocean Sciences, researchers followed up on some earlier work (Requejo and Halpern 1989) and developed procedures for using hopane as an appropriate conserved marker (Prince et al. 1990, Butler et al. 1991). The molecular structure of hopane is presented in Figure 4. Hopane is present in Prudhoe Bay crude oil at about 200 ppm and, though it can be biodegraded, the rate is extremely slow compared to that of other hydrocarbons. It was found sufficiently stable for the purposes of this investigation.

Hopane analysis is quite expensive (up to $2500/sample) and involves a number of analytical steps:

Extraction of 30g of sediment
Removal of polars by alumina column
Analysis by GC and mass spectrometry

By measuring the residual amounts of classes of hydrocarbon compounds relative to the undegraded hopane, investigators were at last able to track the degradation of chemical species and differentiate between fertilized and unfertilized samples. Exxon scientists spent nearly two years on this effort. Figures 5 and 6 are examples of the differences discerned at a 95% confidence level in the change in total resolvable hydrocarbon and polycyclic aromatic hydrocarbon relative to the stable hopane. A detailed summary of the learnings is available elsewhere (Bragg et al. 1992, 1993; Prince et al. 1993). General findings are summarized as follows:

- Biodegradation rates of three to more than five times above background were indicated for adequately fertilized sediments

- Differences in biodegradation rates resulted mainly from varying levels of nutrients (nitrogen) in sediment pore waters. Even higher rates of biodegradation could probably have been achieved by increasing dosage and frequency of fertilizer application

- Examination of nC18/phytane ratio relative to hopane confirmed that both components were being degraded (Figure 7). Therefore, use of the ratio would have suggested lower rates than were actually achieved

- Extent of prior oil degradation is a significant factor affecting biodegradation rate at any point in time. Biodegradation of highly degraded oil may be limited by availability of the oil to the microorganisms due to high molecular weight and low water solubility. However, such oil is usually biologically inert to other organisms as well.

EXTENSION TO INLAND SPILL AND DISPOSAL SITES

Prompted by successful and safe application of bioremediation in Alaska, Exxon's scientists and engineers have been actively pursuing opportunities for expanding the science base developed in Alaska to other types of hydrocarbon contamination—in particular to inland sites. Exxon has an extensive site remediation R&D effort underway and is making maximum use of the advances and learnings from the Alaska cleanup in establishing the potential use of biological processes for soil remediation. Exxon's programs are aimed at all facets of a remediation project, as depicted in Figure 8.

Characterization of the site contamination defines the nature and extent of the contamination, soil properties and geological and hydrogeological features of the site. Based on the Valdez experience, proper sampling and analytical protocols are critical. Also, since the scope of the entire project may depend on this step, statistical planning of the sampling phase is crucial in order to establish confidence in the determination of how much oil is present or removed. Since there are often numerous sources of hydrocarbon at a contaminated site, use of markers to track degradation will be very challenging and perhaps impossible. Therefore, careful data collection, preparation and interpretation are required to maximize confidence in the data. Building on the Valdez learnings, Exxon has developed statistically-designed sampling methods or protocols for obtaining representative samples from what can be highly non-homogeneous contamination areas. The consistency of analytical techniques for determining the amount and type of hydrocarbon in contaminated soils has been improved.

The other important front-end activity is risk assessment. Here the objective is to define a technical basis for determining potential health and environmental impacts in order to arrive at appropriate cleanup targets and strategies and avoid errors in technology selection and deployment.

The technology section of an overall program involves soil treatability testing, review of applicable options, development of design correlations and onsite pilot scale testing. Exxon research guidance studies clearly indicate that in situ bioremediation, if successfully implemented has potential to be the lowest cost approach for site restoration. Reliable techniques have been developed for measuring the treatability of contaminated soils in both laboratory and field applications. These have been used to demonstrate that aged heavy hydrocarbons can be bioremediated under controlled conditions both in the laboratory and in field tests.

There are many challenges to applying the Valdez learnings to inland sites as listed on Table 2. The North Slope crude stranded on the beaches in Alaska had a high percentage of hydrocarbon species that degraded at a relatively rapid rate, even two years after the spill. Also, the contamination was from a single source. Site contamination, especially in older manufacturing facilities, often has accumulated from multiple sources over a period of decades and can be highly weathered. These sites may contain high levels of hydrocarbon species which are more resistant to biodegradation.

Contamination depth in Alaska was largely confined to about a foot and in a few areas reached three feet. At some inland sites, contamination has been detected much deeper. The cobble and pebble beaches found in Alaska were for the most part very porous and permeable. Soils are sometimes tightly packed clay, silt and sand.

The combination of permeable beaches and shallow contamination, along with an open, well-flushed system provided a highly effective mechanism for transporting oxygen and nutrients to the contaminated sediments in Alaska. The tidal cycles, fresh water runoff and wave action also removed degradation by-products. Inland sites are essentially closed systems. A key challenge for deep in-situ bioremediation is a reliable delivery system for nutrients and oxygen.

As far as future initiatives are concerned, plans have been formulated for additional bioremediation activities. Investigations are under way into other microbiological operating regimes. Tests at higher temperatures (up to 140°F) are seeking to explore the activity of naturally occurring, thermophilic bacteria. Reduced pH tests are aimed at triggering fungal activity. Studies on how to take advantage of enzymes to attack complex hydrocarbon molecules are also underway. In addition, we are beginning to tackle the challenge of extending the knowledge acquired to deep in-situ bioremediation.

WHAT'S NEXT FOR BIOREMEDIATION OF MARINE SPILLS?

The success of bioremediation in Alaska has generated interest around the world in this technology for remediating oil spills. In fact, nutrient-enriching fertilizers were used to speed up recovery from a 700 ton spill by the tanker Presidente Arturo Umberto Illia at the Puerto Rosales terminal in Argentina in October 1992. To our knowledge, this is only the second application of fertilizers to remediate a tanker spill (Cutter Information Corp 1992).

The Alaska field data provided extensive information on bioremediation performance on the rocky/gravel shorelines found in Alaska. There is evidence that bioremediation is also effective in other types of environments such as sand beaches and salt marshes, but additional work is needed in those environments to fully establish feasibility and quantify the effectiveness as compared to natural degradation. Differences in sediment permeability affect oxygen and nutrient transport and the nature of the microbial population is different. Thus, further work is needed to extend the results from Alaska to other environments. The experience gained by Exxon, including pioneering work on internal markers, by which changes in oil composition can be traced and used to quantify bioremediation effectiveness, should directly apply to programs in other environments.

The U.S. Marine Spill Response Corporation held a workshop in Washington, D.C., on January 10-11, 1993 to consider the research and development needs associated with the advancement of bioremediation as a tool for marine oil spill response. The meeting was attended by representatives of the EPA, Exxon, Environment Canada, the University of Louisville, NOAA, Amoco Oil Company, University of Cincinnati, NETAC, Texas General Land Office, and the Canadian Department of Fisheries and Oceans. Participants of the workshop considered the spectrum of bioremediation research topics and assembled a list of priority R&D issues. These issues were ranked by each participant following the workshop. The rankings based on a weighted distribution resulted in the following priority areas (ranked in terms of decreasing priority):

1. Factors and criteria that can be used to determine when bioremediation is an appropriate response;

2. Conditions required for effective use of bioremediation and how these conditions are achieved;

2. Conditions required for effective use of bioremediation and how these conditions are achieved;

3. Standards (protocols) for conducting field studies on bioremediation;

4. Endpoints of bioremediation: when to stop;

5. Economical measures of bioremediation rates;

6. Adverse side effects of bioremediation within specific habitats;

7. Feasibility and effects of coupling bioremediation and dispersants;

8. Spatial and temporal fates of nutrients following bioremediation;

9. Critical factors controlling and limitations of EPA/NETAC test protocols and results.

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TABLE 1
COMPOSITIONS OF TWO FERTILIZERS USED IN LARGE-SCALE APPLICATIONS

Inipol EAP22 Oleophilic Liquid

Components	Chemical Formula	Nutrient Concentrations	
Oleic Acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO\ OH$	(N) Nitrogen	7.4%
Tri(laureth-4)Phosphate	$[C_{12}H_{25}(OC_2H_4)_30]_3PO$	(P) Elemental Phosphorus	0.7%
2-Butoxyethanol	$HO–C_2H_4–O–C_4H_9$		
Urea	$NH_2–CO–NH_2$		
Water	H_2O		

Customblen Granular Solid

Components	Chemical Formula	Nutrient Concentrations	
Ammonium Nitrate	NH_4NO_3	(N) Nitrogen	28.0%
Calcium Phosphate	$Ca_3(PO_4)_2$	(P) Elemental Phosphorus	3.5%
Ammonium Phosphate	$(NH_4)_3PO_4$		

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FIGURE 1
GRAVIMETRIC OIL LOSS FROM COBBLE SURFACE SEDIMENT IN PASSAGE COVE TESTS

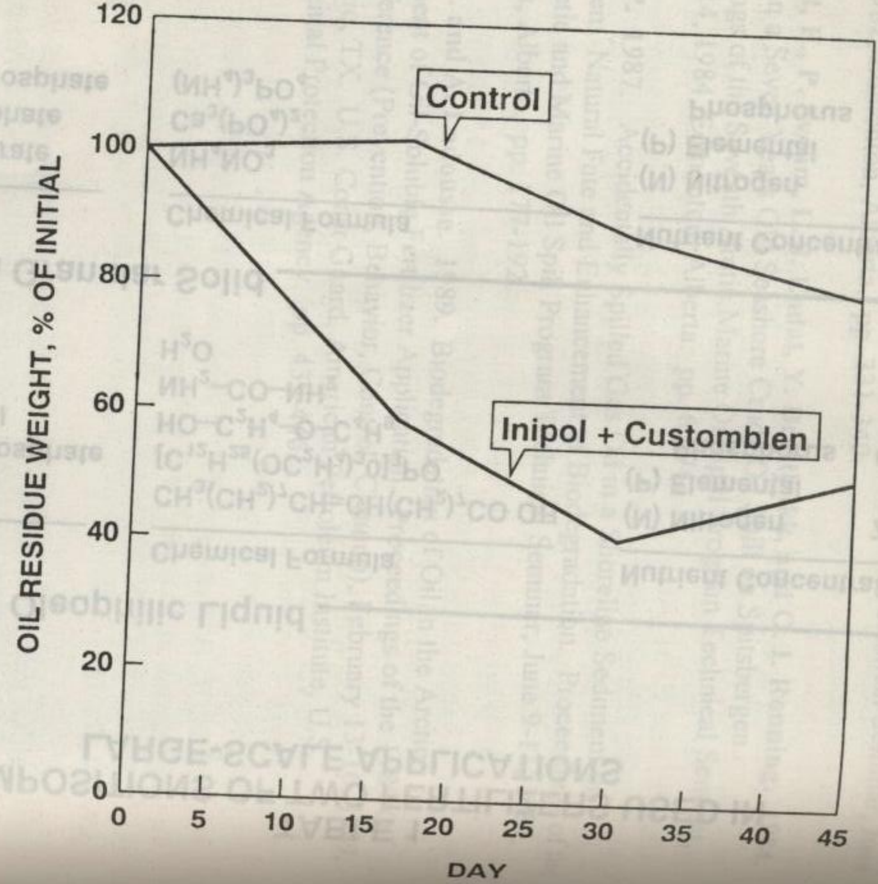

FIGURE 2
MAP OF SUMMER 1990 MONITORING SITE KN-135

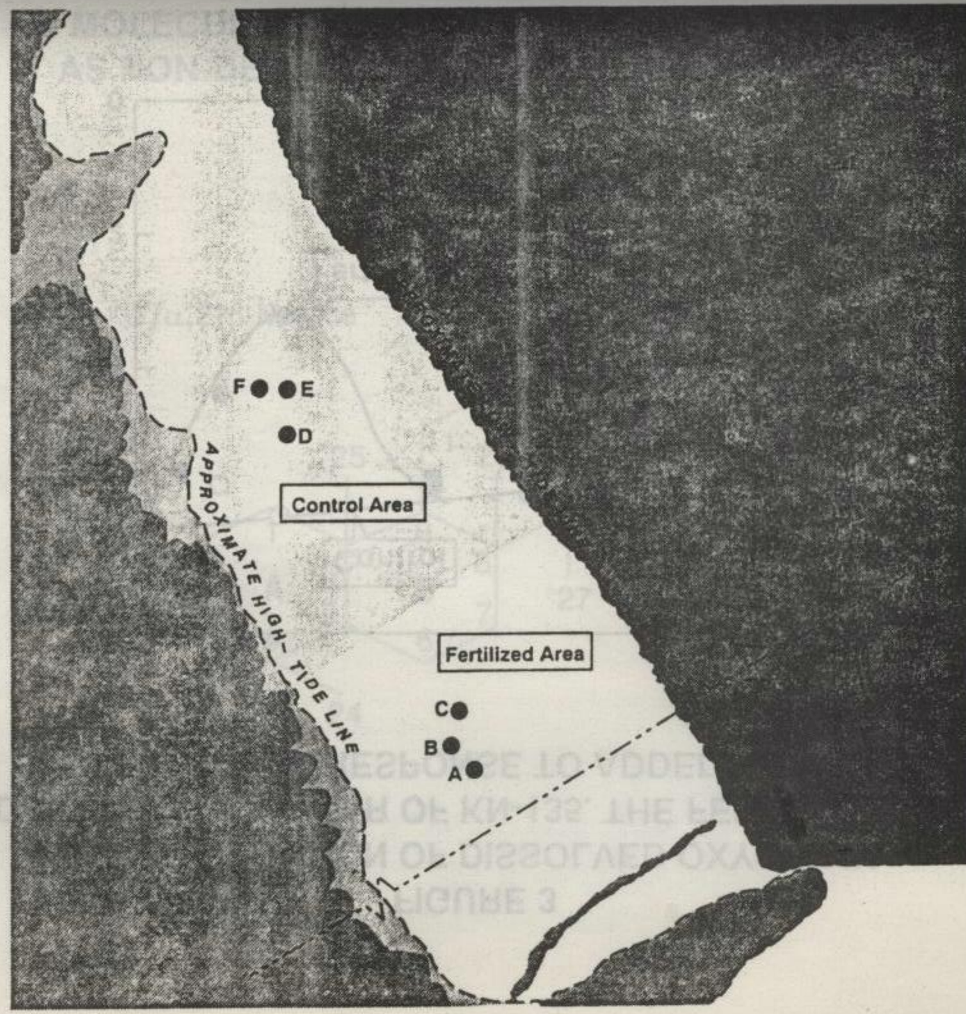

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FIGURE 3

CONCENTRATION OF DISSOLVED OXYGEN IN SEDIMENT PORE WATER OF KN-135. THE FERTILIZED PLOT SHOWED CLEAR RESPONSE TO ADDED NITROGEN.

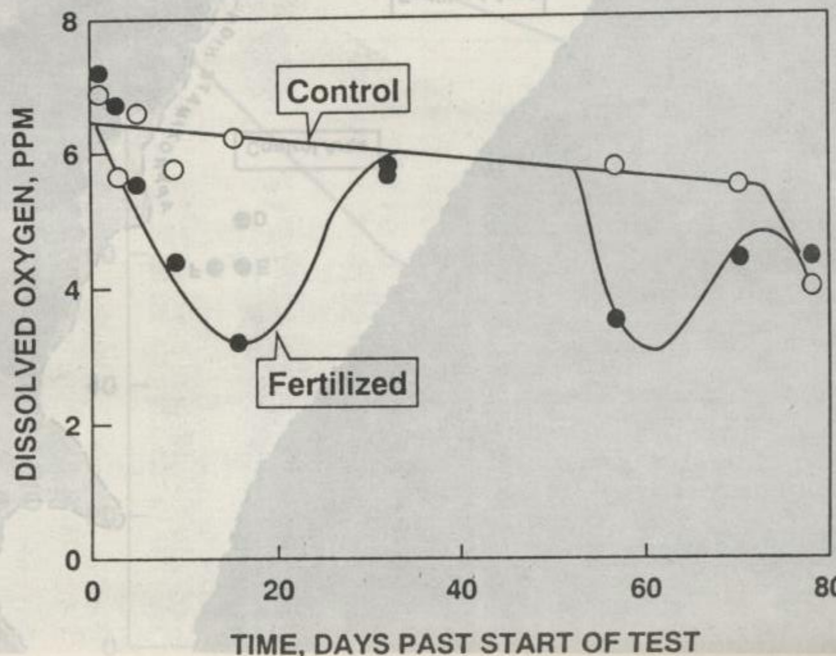

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FIGURE 4
MOLECULAR STRUCTURE OF THE HOPANE USED AS NON-DEGRADED MATERIAL BALANCE TRACER

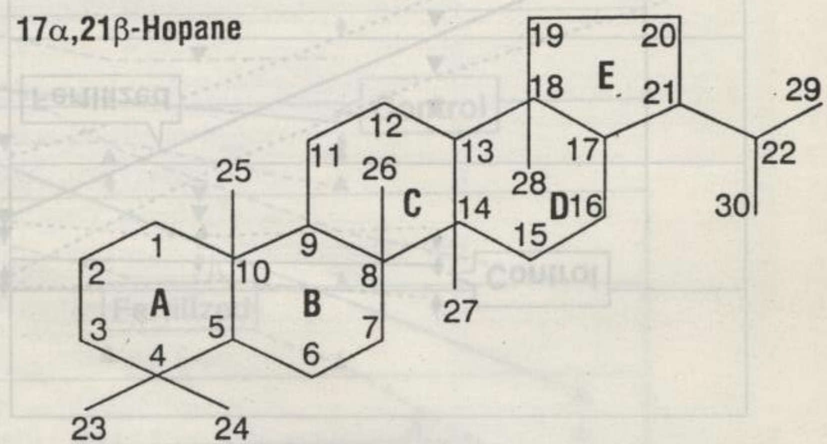

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FIGURE 5

CHANGE IN TOTAL RESOLVABLE HYDROCARBON/HOPANE IN FERTILIZED VS. CONTROL SEDIMENT, KN-135 SUBSURFACE

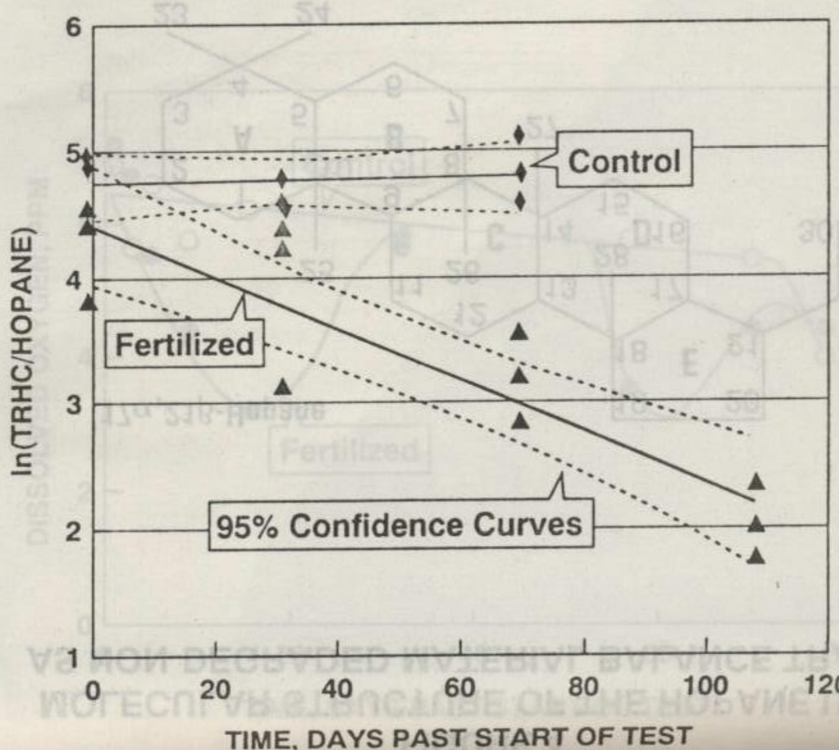

FIGURE 6
CHANGE IN TOTAL POLYCYCLIC AROMATIC HYDROCARBONS/HOPANE IN FERTILIZED VS. CONTROL SEDIMENT, KN-135 SUBSURFACE

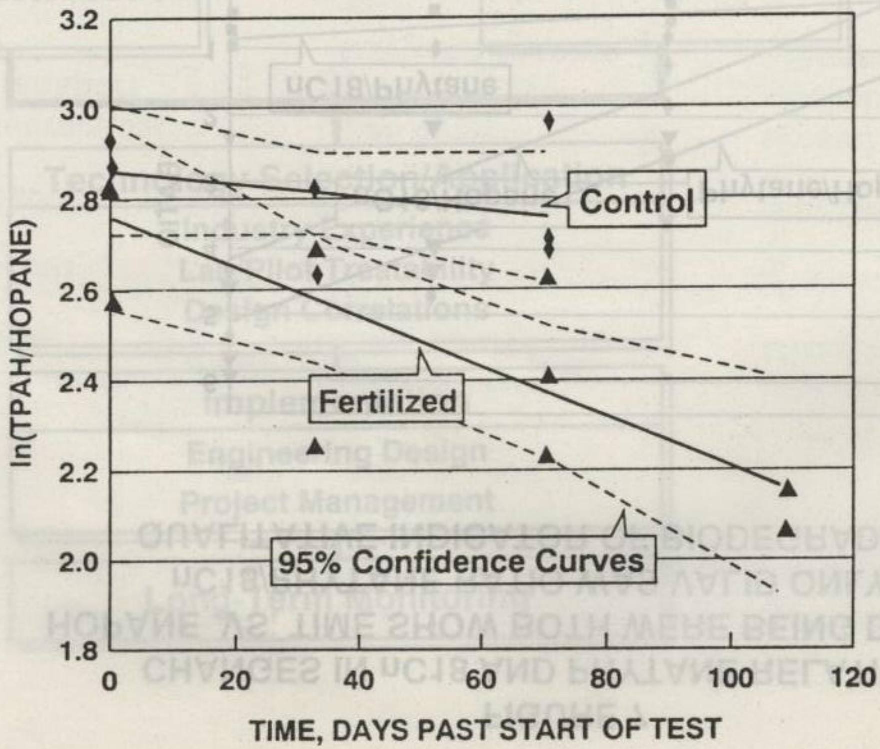

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FIGURE 7

CHANGES IN nC18 AND PHYTANE RELATIVE TO HOPANE VS. TIME SHOW BOTH WERE BEING DEGRADED; nC18/PHYTANE RATIO WAS VALID ONLY AS QUALITATIVE INDICATOR OF BIODEGRADATION

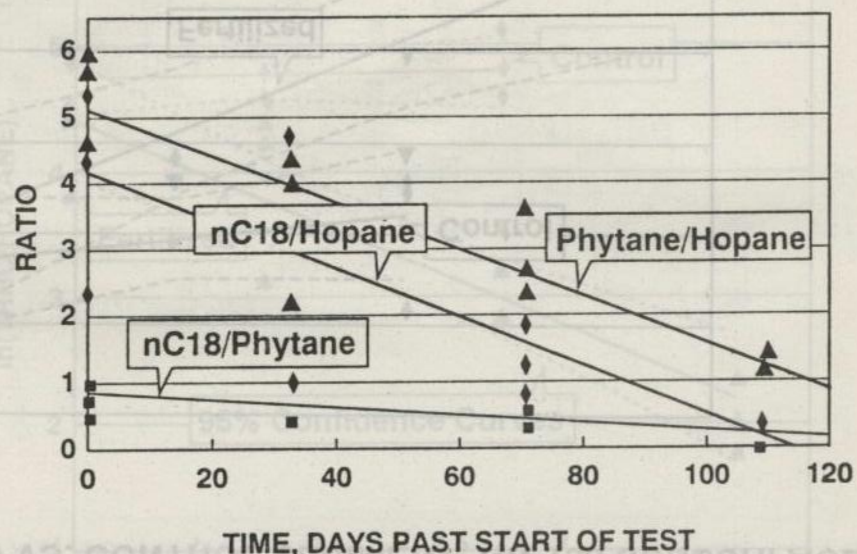

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FIGURE 8
OVERVIEW OF A SITE REMEDIATION PROJECT

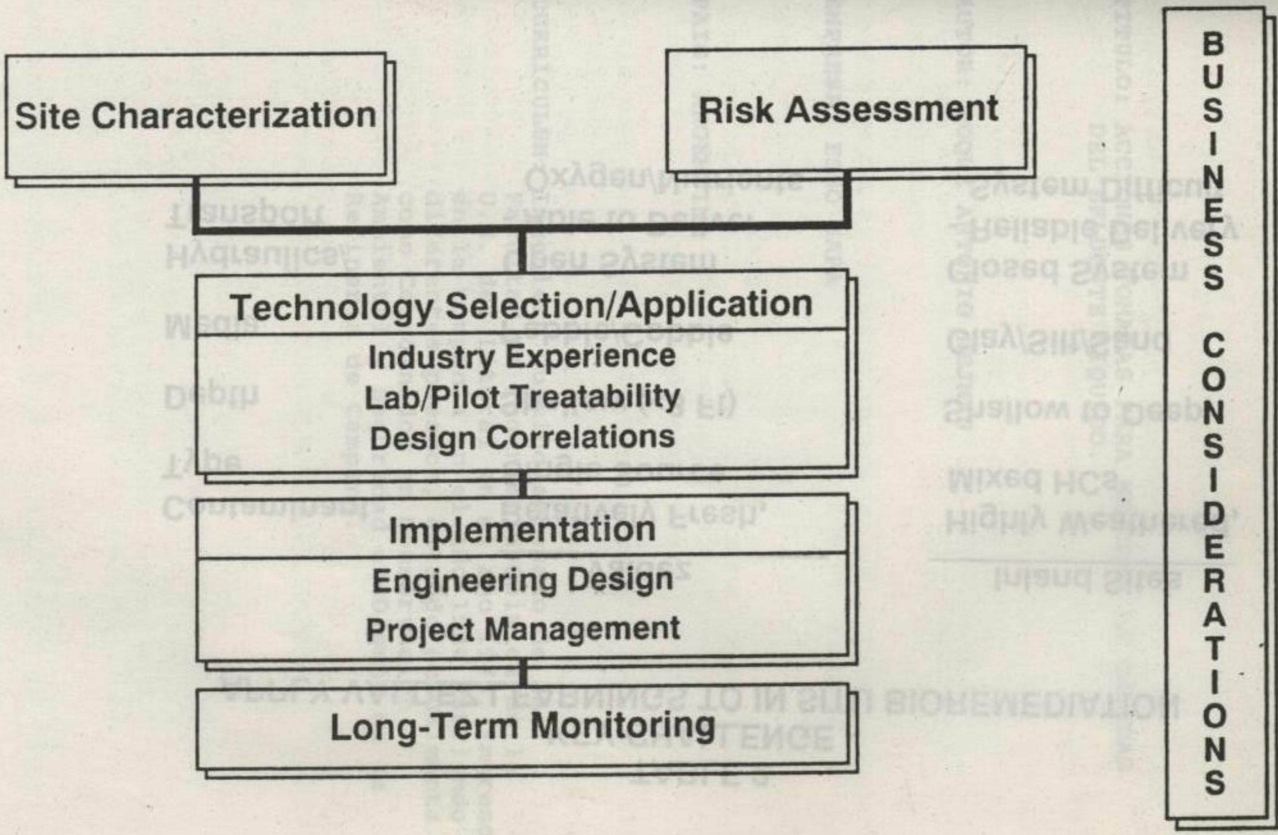

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TABLE 2
KEY CHALLENGE -
APPLY VALDEZ LEARNINGS TO IN SITU BIOREMEDIATION

	Valdez	Inland Sites
Contaminant Type	Relatively Fresh, Single Source	Highly Weathered, Mixed HCs
Depth	Shallow (<3 Ft)	Shallow to Deep,
Media	Pebble/Cobble	Clay/Silt/Sand
Hydraulics/ Transport	Open System - Able to Deliver Oxygen/Nutrients	Closed System - Reliable Delivery System Difficult

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