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Bioremediation as a technology: Experiences with the Exxon Valdez oil spill*

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Abstract

The results from our oil spill bioremediation project have demonstrated convincingly that fertilizers can be applied to oiled beaches to overcome nutrient limitations, thereby enhancing biodegradation of the oil. In Prince William Sound, the natural biodegradation rate of oil on the beaches was found to be quite high, primarily because of small concentrations of ammonia and phosphate in seawater that are introduced into the beach material with each tide. However, the addition of fertilizers was capable of increasing this biodegradation as much as two to three fold above background activity. In addition, the extent of enhanced degradation was such that beaches became visually cleaner and aesthetically improved.

Introduction

Several weeks following the Exxon Valdez oil spill in Prince William Sound, Alaska, several million gallons of Prudhoe Bay crude oil, a well studied oil with respect to previous cold water biodegradation studies [1–5], had contaminated almost 300 miles (480 km) of rocky coast line in Prince William Sound. This confronted Exxon, the State of Alaska, and the U.S. Coast Guard with the largest clean-up effort in U.S. history. As a variety of clean-up options were assessed and implemented, it became clear to EPA's Office of Research and Development and its scientists that bioremediation was also a reasonable clean-up option despite the complexity of the environmental setting. We reasoned that the oil would become quickly colonized with oil degrading bacteria but that their ability to degrade oil would be limited by the availability of nitrogen and phosphorous nutrients. Artificially adding these nutrients would therefore enhance biodegradation rates, something that has been observed many times in laboratory studies [6–12]. Thus, the Alaskan Bioremediation Project was initiated. Accounts of this effort have been previously published [13–19]. This

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The existence of a large EPA research program in bioremediation [20] provided the backbone for the bioremediation project. An approach was developed to determine if the addition of nitrogen and phosphorous-containing fertilizers to oil-contaminated beaches would sufficiently enhance oil biodegradation rates to permit consideration of bioremediation as a secondary clean-up tool. A plan was conceived to conduct an initial field demonstration of this approach and then if successful, recommendations for wider scale application would be made to Exxon. The Environmental Protection Agency would then provide a follow up field study as a definitive indication of the success of the large scale application.

A team of experts from the different research laboratories within EPA's Office of Research and Development (ORD), including on site contractors were assembled to implement the field demonstration. It was the combined effort of this team that set the stage for the overall success of the project. In addition, Exxon generously provided, through a Technology Transfer Cooperative Agreement, financial and operational support [13], without which the project would not have been possible.

The field demonstration

Field operations were begun in early May 1989, using the mobilization capability of ORD laboratories at Las Vegas, NV; Gulf Breeze, FL; Cincinnati, OH; Athens, GA; Research Triangle Park, NC; and Ada, OK. Two sites were selected, Snug Harbor and Passage Cove [16,17]. These beaches were comprised mainly of large cobblestone overlying a mixed sand and gravel base. The Snug Harbor beaches had a moderate degree of oil contamination confined to a broad band within the intertidal zone. At the initiaion of the project, beaches that had been physically washed by the Exxon process (high pressure water jets and water heated to 140°F or 60°C) to physically force the oil off the beach surface were not yet available for testing. Thus, the Snug Hargor study site was chosen to model beach conditions following cleanup. Passage Cove was a heavily oiled beach that was physically washed by Exxon, removing the bulk oil and spreading the remaining oil over beach surfaces. Both beaches had a thin layer of oil covering the surface of the cobblestone, as well as oil mixed into the sand and gravel under the cobble to varying depths.

Selection of fertilizers, was based on considerations of application strategies, logistical problems for large scale application, commercial availability (particularly if large scale application became reasonable) and the ability to provide nitrogen and phosphorous nutrients to the microbial communities on the surface and the subsurface beach material over sustained periods. Three application strategies were adopted for testing; commercially available slow release formulations, an oleophioic fertilizer, and water soluble fertilizer applied as a solution [17].

Commercial slow release fertilizer formulations were screened for the best nutrient release rate characteristics. The strategy was to apply the best product to the beach surface and then allow tidal action to disperse the released nutrients over the contaminated area of the beach. The product had to remain on the beach for several weeks while still delivering sufficient quantities of nutrients. Fertilizer granules (about 2–3 mm in diameter), produced by Sierra Chemicals (Milpitas, CA), were selected as the main slow release fertilizer formulation. These granules (Customblen) have a N:P:K ratio of 28:8:0 and slowly release ammonia, nitrate and phosphate from inorganic ammonium nitrate and ammonium phosphate encapsulated inside a diene-treated vegetable oil coating. The fertilizer granules were broadcast on to the beach surface at a surface concentration of 90 g/m^2 using a mechanical seed spreader. Their high specific gravity, propensity to adhere to the oil and their tendency to entrain under rocks and in interstitial spaces, assured that they would remain on most low and moderate energy beaches in Prince William Sound for two to three weeks.

Oleophilic fertilizers are thought to essentially dissolve the nutrients into the oil by applying the material directly on the oiled beach material. Nutrients sequestered in the oil phase would presumably facilitate bacterial growth on the surface over sustained periods. The oleophilic fertilizer Inipol EAP 22, produced by Elf Aquataine Company (Artix, France) was selected. It was the only commercially available fertilizer of this type that could be produced in large quantities on short notice. This product is a stable microemulsion consisting of a core of urea (the nitrogen source) surrounded by an oleic acid carrier. Lauryl phosphate (a surfactant and the source of phosphorous) is incorporated as a stabilizer. Application in Prince William Sound was conducted using a backpack sprayer to give a thin coating over the oiled beach material at a concentration of approximately 0.1 gallon (0.38 L/m^2).

The third type of fertilizer application involved spray irrigation with an aqueous fertilizer solution. This approach produced the most defined, controlled and reproducible introduction of nutrients into the oiled beach material, particularly for oil below the beach surface. It was accomplished by dissolving commercially available sources of ammonium nitrate (NPK 34:0:0) and triple phosphate (NPK 0:45:0) into seawater pumped from below the beach. The resulting fertilizer solution was then sprayed over the beach surface at low tide using a pump and lawn sprinkler heads. The fertilizer application rate was 0.4 inches (1 cm) of water applied over a four hour period, giving an approximate concentration of 6.9 g of N/m^2 and 1.5 g P/m^2.

The first application of the oleophilic fertilizer occurred July 8, 1989 at the Snug Harbor site. Approximately 2–3 weeks following application, the treated beach showed a visually pronounced reduction in the amount of oil on the surface of the cobblestone. This produced a striking "window" against the oiled beach background (Fig. 1) . Differences between treated and untreated portions of the beach were dramatic. Close examination of the beach, however, showed that significant quantities of oil remained under the cobblestone as well as within the beach subsurface. Eventually, even this oil disappeared slowly over the next few weeks. This contrasted with the untreated control areas, in which there was little visual change. Subsequent studies in the laboratory verified that Inipol was not a chemical rock washer.

Definitive information on the role of biodegradation in this event, was established by extracting oil from surface samples of cobble in the oleophilic fertilizer-treated beach and analyzing the extracts by gas chromatography. The sampling and analysis showed the this visual disappearance of the oil was accompanied by significant decreases in total oil residues (i.e., weight of extractable material) and changes in hydrocarbon composition. Gas chromatographic profiles of straight chain aliphatic hydrocarbons in samples from treated and control beaches at time zero and 4 weeks following fertilizer application are shown in Figs. 2 and 3. Clearly there are large differences in the shape of the profile and the concentration of individual hydrocarbons (peak heights) that are associated with fertilizer application. This change in hydrocarbon composition was largely due to biodegradation,. Thus, it is reasonable to assume that the decreases in oil residue weight on the cobblestone was caused by biodegradation. We suspect that after a cerain extent of oil biodegradation was achieved, the physical nature of the oil changed into a less sticky, flaky consistency and this innocuous degraded material was then easily scoured from the rock surfaces by tidal action.

An ecological monitoring program established in Snug Harbor to check for potential adverse ecological effects further verified the safety of bioremediation as a clean-up tool [19]. The potential for eutrophication was determined by measurements of ammonia, phosphate and chlorophyll concentrations, bacterial numbers, and primary productivity in the water column directly offshore of the fertilizer-treated beaches and in control areas distant from the test plots. Measurements in the experimental area were not significantly different from the range of values observed in control areas. In addition, ammonia, the only component of the fertilizers acutely toxic to marine animals (based on laboratory bioassays with indigenous and surrogate test species), never exceeded toxic concentrations. Mussels suspended in floating cages just offshore from the treated beaches, showed no bioaccumulation of oil residues, strongly supporting the contention that fertilizer addition did not cause the release of undegraded oil from the beaches. Finally, genotoxicity assays ameliorated by bioremediation and no genotoxic degradation products were formed.

Results from our initial field studies were sufficient for Exxon to consider the use of bioremediation on a large scale as a finishing step for their clean-up effort. We recommended that the oleophilic fertilizer, Inipol, be applied to

Fig. 1. Oil contaminted beach in Snug Harbor two weeks following application of oleophilic fertilizer. Area to the right is treated; area to the left is untreated.

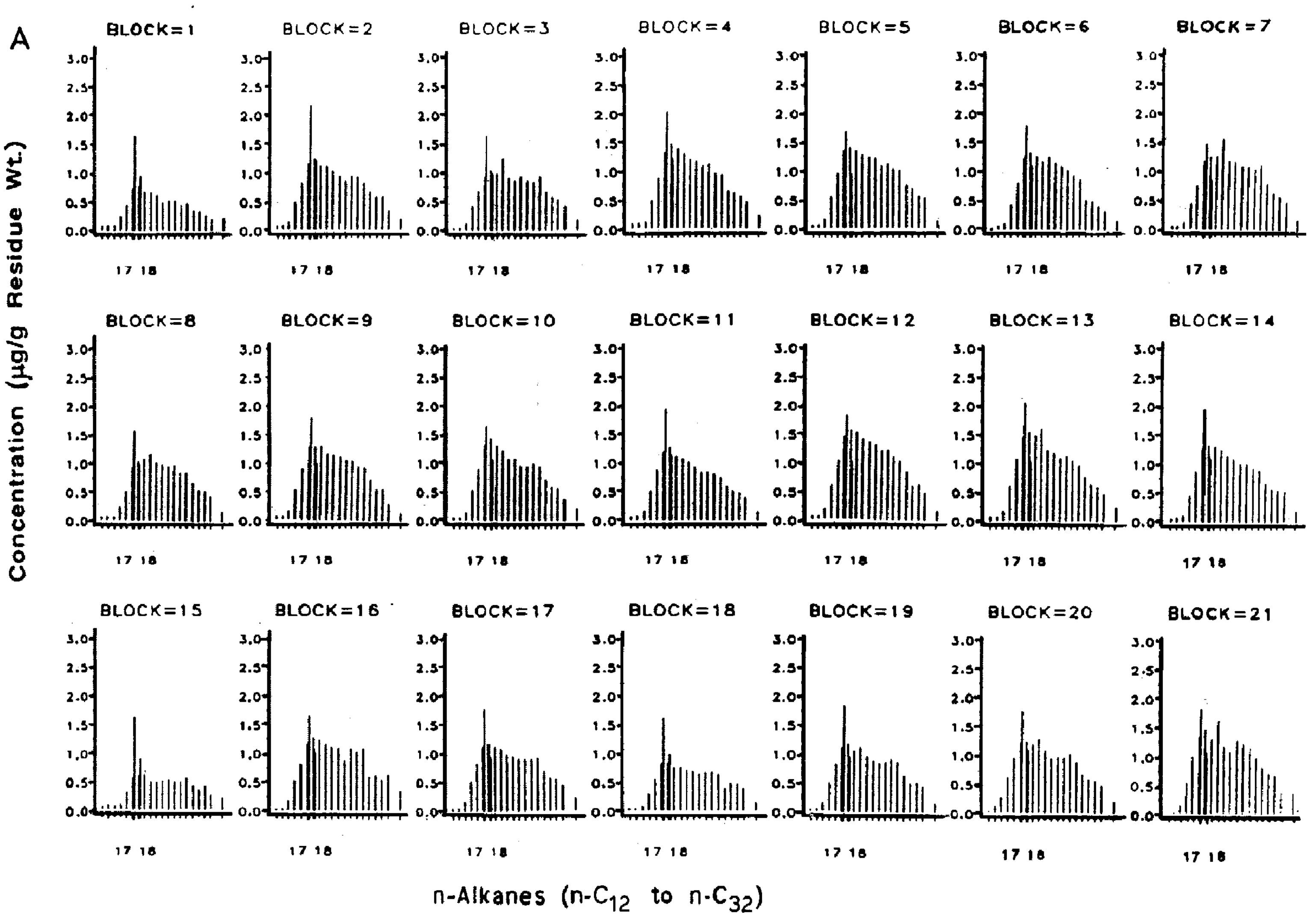

Fig. 2 (a). Recreated gas chromatographic profiles from samples of oil extracted from the mixed sand and gravel under the cobble prior to application of oleophilic fertilizer at Snug Harbor. Blanks indicate data not available. (Note that all concentrations are on the same scale.)

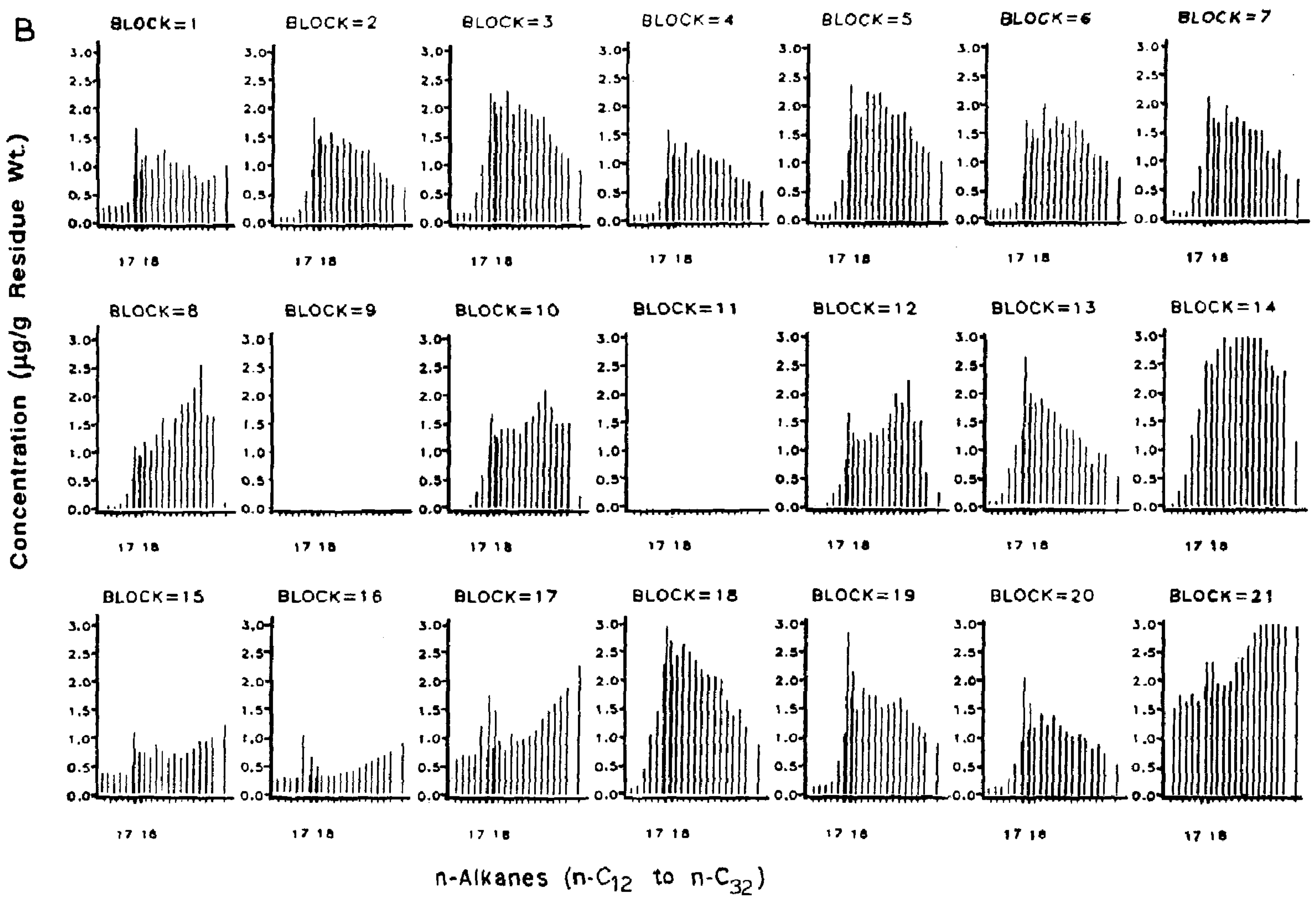

Fig. 2(b). Recreated gas chromatographic profiles from samples of oil extracted from the mixed sand and gravel under the cobble two weeks following application of oleophilic fertilizer at Snug Harbor. Blanks indicate data not available. (Note that all concentrations are on the same scale.)

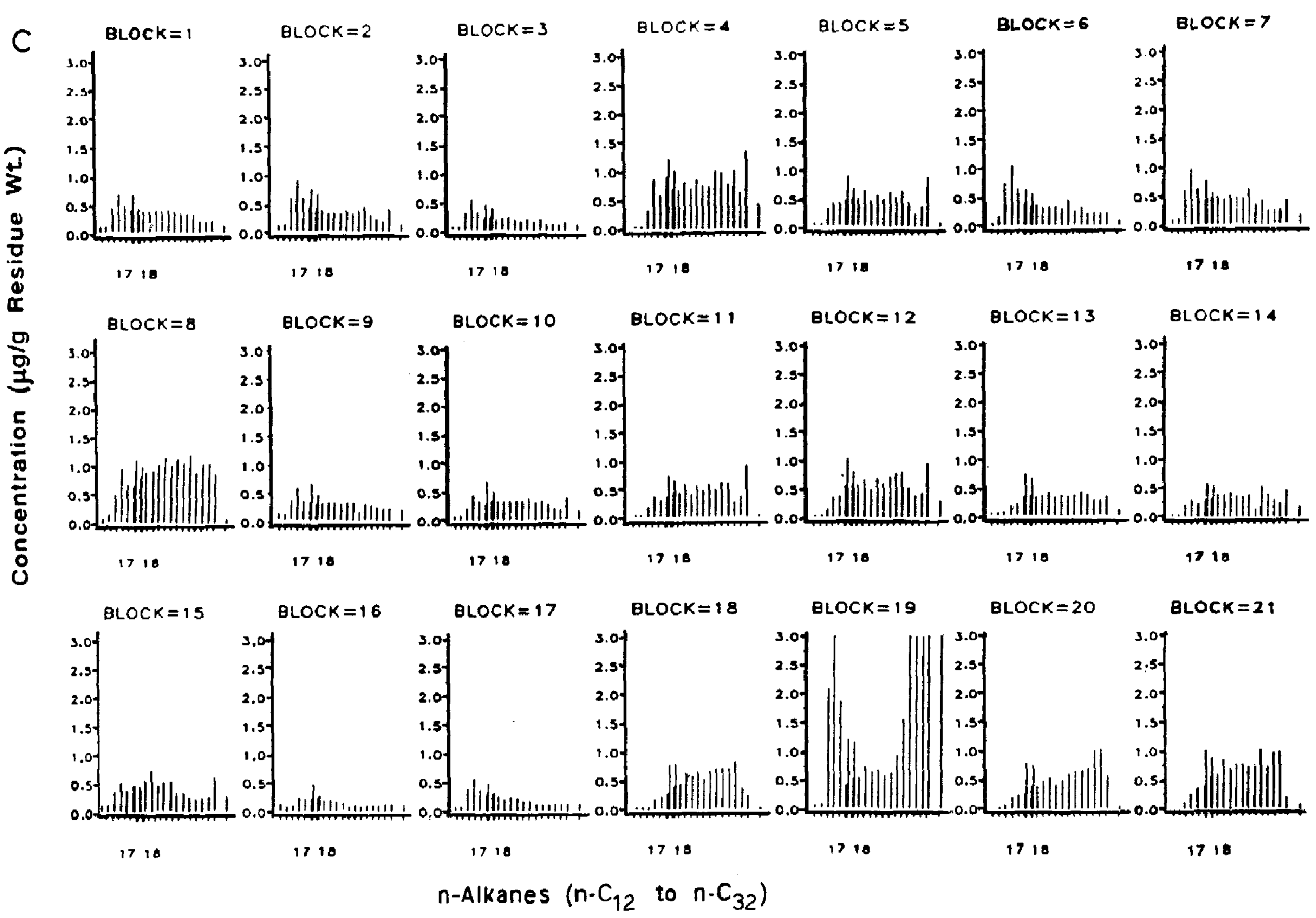

Fig. 2(c). Recreated gas chromatographic profiles from samples of oil extracted from the mixed sand and gravel under the cobble four weeks following application of oleophilic fertilizer at Snug Harbor. Blanks indicate data not available. (Note that all concentrations are on the same scale.)

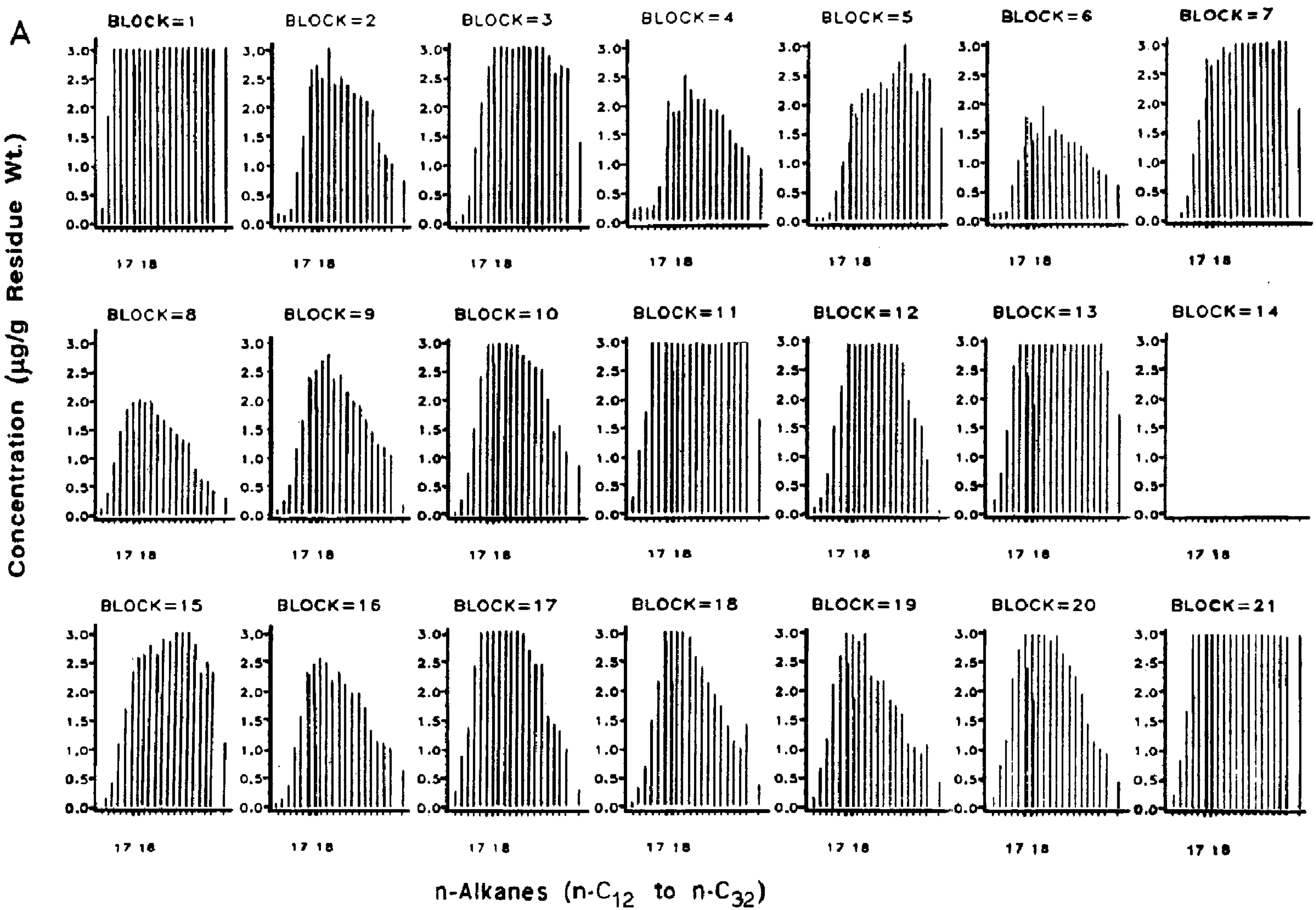

Fig. 3(a). Recreated gas chromatographic profiles normalized to oil residue weight from samples of oil extracted from the mixed sand and gravel under the cobble prior to application of fertilizer briquettes at Snug Harbor. Blanks indicate data not available. (Note that all concentrations are on the same scale.)

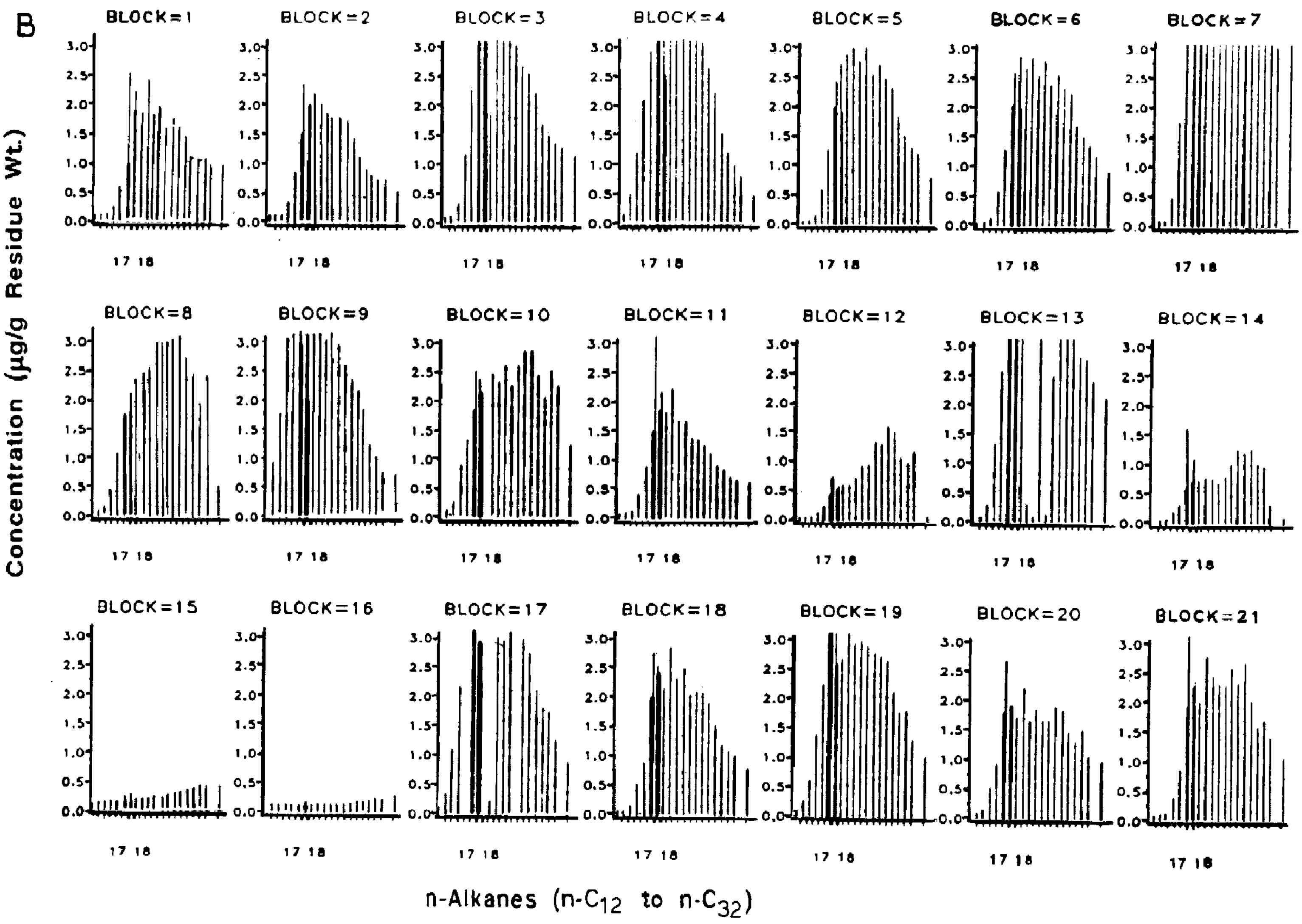

Fig. 3(b). Recreated gas chromatographic profiles from samples of oil extracted from the mixed sand and gravel under the cobble two weeks following application of fertilizer briquettes at Snug Harbor. Blanks indicate data not available. (Note that all concentrations are on the same scale.)

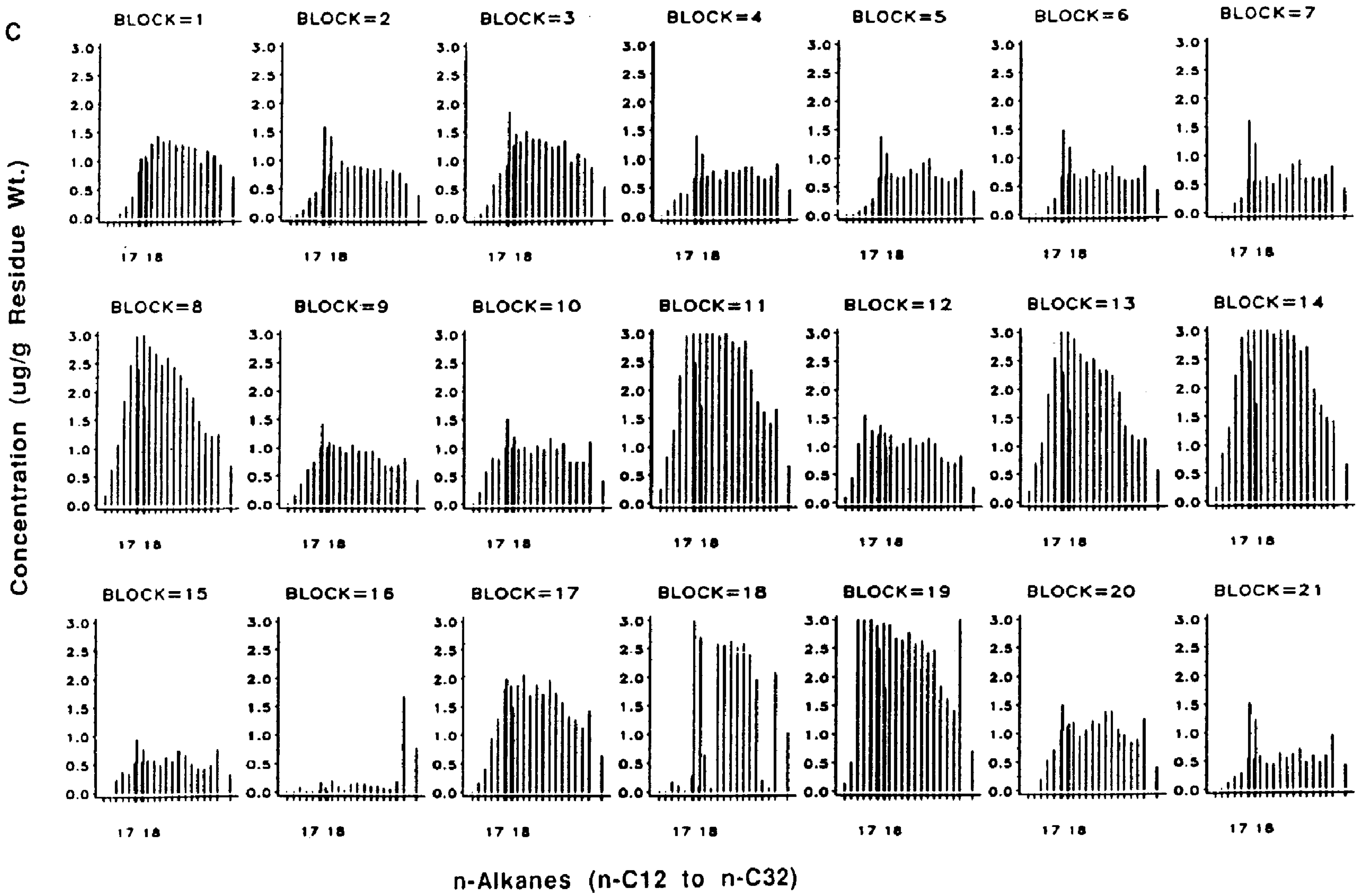

Fig. 3(c). Recreated gas chromatographic profiles from samples of oil extracted from the mixed sand and gravel under the cobble four weeks following application of fertilizer briquettes at Snug Harbor. Blanks indicate data not available. (Note that all concentrations are on the same scale.)

beaches with only surface oil and that a combination of Inipol and the fertilizer granules (Customblen) be applied to beaches where there were both surface and subsurface oil found. The granules provided a simple means of releasing nutrients into the beach subsurface by tidal action and thereby potentially enhancing biodegradation of subsurface oil.

Exxon began fertilizer application in early August 1989, to approximately 50 miles (80 km) of beach in Prince William Sound that had been physically washed. Increasing the biodegradation rate of oil at this point was very important because maximal degradation could be achieved before winter conditions slowed biodegradation processes. In many cases, the results of large scale fertilizer application were as dramatic as our initial observations at Snug Harbor; that is, where the oil was spread tinly over the cobble surface (as was the case on many beaches that had been physically washed), the oil disappeared over a 20-day period. Unexpectedly, it also appeared that even oil underneath the cobble had disappeared in a shorter time period than observed at Snug Harbor. Although it is difficult to prove experimentally, we believe that the physical cleaning process used by Exxon dispersed the oil throughout the beach material to such an extent that the exposed oil surface area was greatly increased, allowing greater bacterial colonization and subsequent biodegradation.

The Passage Cove study was initiated in late July, 1989, as the definitive technical support site for the large scale application of Inipol and Customblen fertilizers. These fertilizers were applied in combination to a large test beach and samples of beach material were analyzed for changes in oil residue weight and aliphatic hydrocarbon composition as before. These changes were compared to those observed in an untreated control beach. In addition, a beach treated with a seawater solution of inorganic fertilizer (applied via a sprinkler system) was examined in the same way.

Approximately 2 to 3 weeks following initiation of this study, oil on the cobble surfaces in the Customblen/Inipol and fertilizer solution-treated beaches, had been degraded to the point of producing visibly cleaner surfaces much as we had seen in Snug Harbor. Surface oil on the control beach, however, was still very apparent showing no visual reduction in the amount of oil. Disappearance of oil from the rock surfaces on the beach treated with the fertilizer solution provided definitive proof that biodegradation (and not chemical washing) was responsible for the oil removal, as there was no other reasonable mechanism to explain the effect of nutrient addition.

Despite sampling and interpretation complications resulting from the high variability in oil distribution on the beaches, we have been able to show, statistically, that oil biodegradation (as measured by changes in oil chemistry) was significantly greater on the beach treated with the fertilizer solution than it was on the control beach. Based on this information, we projected that after 45 days approximately 4–5 times more oil degradation would have occurred on the solution-treated test beach. This corresponded to an enhanced biodegradation rate of about 2-3 fold. Results were similar on the Inipol/Customblen-treated beach except statistically significant differences were not possible to establish. However, it appeared that accelerated biodegradation (approximately a 2–3 fold increase in biodegradation rates), occurred early in the test when nutrient concentrations were highest.

The striking results of the fertilizer solution application strongly supported the idea that oil degradation in Prince William Sound was nutrient limited. In addition, it implied that fertilizer reapplication (maintaining nutrient concentrations at high levels for long periods) was important. Nutrient monitoring studies on the fertilizer solution-treated beach in Passage Cove showed that low concentrations of ammonia and phosphate accumulated in the beach subsurface and persisted (in the absence of further application) for at least 4–5 days. This nutrient-ladened water than continued to expose oiled beach material to nutrients with each incoming tide.

The long term benefit of fertilizer application was realized during examination of the beaches in Passage Cove in November, 1989 and early June, 1990. Virtually no oil was observed on either of the treated beaches, both at the surface and the subsurface (12" or 30 cm depth). However, the untreated control beach still showed areas of heavy oil contamination in the subsurface beach material. These observations provided the final definitive demonstration of the long term success that can be expected from bioremediation of oil contaminated beaches.

As a result, in spring of 1990, bioremediation became an integral part of a clean-up plan for the remaining oil-contaminated shorelines in Prince William Sound. To follow the success of this treatment, a joint bioremediation monitoring program was conceived and implemented by scientists from Exxon, EPA, ADEC and the University of Alaska (using logistical and resources support from Exxon). Several beaches where fertilizer application was underway were selected and monitored for increases in oil degrading microbial activity and oil degrader biomass, relevant changes in oil chemistry, and indications adverse ecological effects. Much of the emphasis centered on the ability of bioremediation to work effectively on oil in the beach subsurface (0.3–1.0 m depths).

The monitoring program demonstrated that fertilizer addition stimulated oil biodegradation activities by 3–4 fold and that the biodegradation was affecting the removal of more than just the easily degradable aliphatic hydrocarbons from the oil. In addition, the observed sustained levels of nitrogen and phosphorous in interstitial water corresponded with stimulation of microbial activities in the beach subsurface to a depth of at least 70–80 cm. Again there was a total absence of any measurable adverse ecological effects. The success of this monitoring program paved the way for multiple reapplication of the fertilizers, a necessary step in many cases because of large quantities of oil remaining in some area.

Summary and conclusions

The results from our oil spill bioremediation project have demonstrated convincingly that fertilizers can be applied to oiled beaches to overcome nutrient limitations, thereby enhancing biodegradation of the oil. In Prince William Sound, the natural biodegradation rate of oil on the beaches was found to be quite high, primarily because of small concentrations of ammonia and phosphate in seawater that are introduced into the beach material with each tide. However, the addition of fertilizers was capable of increasing this biodegradation as much as two to three fold above background activity. In addition, the extent of enhanced degradation was such that beaches became visually cleaner and aesthetically improved.

Our work also showed that the most effective means to enhance oil biodegradation was to insure that the oil degrading microbial communities were exposed to high concentrations of nutrients for a sustained periods of time (days to weeks). An oleophilic fertilizer appeared to be very effective in this regard and it's application was straight forward. The long term positive effect of this fertilizer application suggests that nutrients are sequestered at the oil–water interface in such a way that biodegradation is promoted. When used in combination with a slow release fertilizer granules, subsurface oil was also degraded to a greater extent compared to untreated reference beaches. Fertilizer solutions were the most effective nutrient application technique in terms of rate and extent of oil degradation but they required a more complicated application approach.

Bioremediation of oil-contaminated beaches was shown to be a safe clean-up technology as no adverse ecological side effects were observed i.e. no eutrophication, no acute toxicity to sensitive marine test species, and no release of undegraded oil residues from the beaches occurred.

The success of our field demonstration program has now set the stage for the consideration of bioremediation as a key component in any clean-up strategy developed for future oil spills. Its use and effectiveness will depend on the amount of oil present in the contaminated environmental matrix; i.e., a longer time will be required for degradation of high concentrations of oil and consequently a longer period of fertilizer application will also be required. In addition, location of the oil (in the absence of physical clean-up, subsurface oil may only be treatable by bioremediation) and the acceptablility of other clean-up options must be considered. In most aquatic environments, enrichments of oil degrading microbial communities occur relatively soon after oil contaminates shorelines. It is unlikely that natural sources of nitrogen and phosphorous will be sufficient to give maximal degradation rates in light of the available degradable organic carbon from the oil. Thus, the application of fertilizers should enhance degradation and eventually remove the oil. Although oxygen may become limiting in certain situations (e.g. for fine grain sandy beaches) the high porosity and large tidal fluxes characteristic of Prince William Sound beaches precluded this as a limitation.

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