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Efficacy of commercial inocula in enhancing biodegradation of weathered crude oil contaminating a Prince William Sound beach

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SUMMARY

In a laboratory study evaluating the effectiveness of 10 commercial products in stimulating enhanced biodegradation of Alaska North Slope crude oil, two of the products provided significantly greater alkane degradation in closed flasks than indigenous Alaskan bacterial populations supplied only with excess nutrients. These two products, which were microbial in nature, were then taken to a Prince William Sound beach to determine if similar enhancements were achievable in the field. A randomized complete block experiment was designed in which four small plots consisting of a no-nutrient control, a mineral nutrient plot, and two plots receiving mineral nutrients plus the two products were laid out in random order on a beach in Prince William Sound that had been contaminated 16 months earlier from the Exxon Valdez spill. These four plots comprised a 'block' of treatments, each block being replicated four times on the same beach. Triplicate samples of beach sediment were collected at four time intervals and analyzed for oil residue weight and alkane hydrocarbon profile changes. The results indicated no significant differences ($P < 0.05$) among the four treatments in the 27-day time period of the experiment. A statistical power analysis, however, revealed that the variability in the data prevented a firm conclusion in this regard. Failure to detect significant differences was attributed not only to variability in the data but also to the highly weathered nature of the oil and the lack of sufficient time for biodegradation to take place.

INTRODUCTION

The largest field bioremediation test ever attempted was conducted by the U.S. Environmental Protection Agency (EPA) and the Exxon Corporation on the shorelines of Prince William Sound, Alaska, following the oil spill from the supertanker Exxon Valdez in March, 1989 [12]. From the results of that study, investigators concluded that application of nitrogen and phosphorus nutrients enhanced biodegradation of the crude oil. Furthermore, no adverse environmental effects were observed as a result of the fertilizer application.

In addition to application of nutrients, bioremediation in the field may be enhanced by inoculation with allochthonous microorganisms. Cultures and cultural products have been added to different environments to stimulate or enhance biological removal of contaminants. Some of the investigations have demonstrated enhancement of biodegradation by this means, while others failed to demonstrate such enhancement [10]. In a recent study, Dott

et al. [7] compared fuel oil degradation rates of activated sludge microorganisms with nine different commercial bacterial cultures in separate laboratory flasks. The rate and extent of *n*-alkane and total hydrocarbon degradation by the diverse populations in activated sludge were significantly higher than any of the highly adapted commercially available cultures. Lehtomaki and Niemela [11] found that addition of brewers' yeast to oil-contaminated soil enhanced oil removal by factors of 2- to 10-fold. This was most likely due to the supply of critical nutrients, vitamins, or cofactors lacking in the soil naturally. Christianson and Spraker [5] reported a series of case histories of refinery wastewater treatment plants using commercial cultures to overcome various problems, such as foaming, toxic loads, low biomass, etc. Most success with biodegradation enhancement by allochthonous microbial cultures has been achieved when chemostats or fermentors were used to control conditions or reduce competition from indigenous microflora [19].

Venosa et al. [17] recently conducted laboratory tests on ten commercial products (eight bacterial inocula, one dispersant, and one fertilizer formulation) claimed by the vendors to be able to enhance microbial degradation of weathered crude oil. The products were selected from a public solicitation by EPA and review of proposals by a

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panel of experts convened by the National Environmental Technology Applications Corporation (NETAC), a non-profit organization dedicated to the commercialization of environmental technologies. Laboratory tests on the products were conducted to measure the rate and extent of oil degradation in closed ecosystems. Weathered oil from an Alaskan beach, contaminated by oil from the Exxon Valdez spill, and seawater from Prince William Sound were used in the tests. The NETAC panel reviewed the results of the tests and agreed on the recommendation for field testing two of the products that exceeded the performance of inorganic nutrient addition alone. This paper presents the results of the field testing of the two selected products on a beach in Prince William Sound. The objective was to determine if commercial microbiological products were able to enhance bioremediation of an oil-contaminated beach to an extent greater than that achievable by simple fertilizer application. The two companies that participated in the testing were Sybron, Inc. and ERI-Waste Microbes, Inc.

MATERIALS AND METHODS

Site description

The site selected for the field testing was located on Disk Island (latitude 60° 30' N, longitude 147° 40' W), designated DI-67A by the National Oceanic and Atmospheric Administration. Disk Island is located between Ingot Island and the northeast tip of Knight Island in Prince William Sound (Fig. 1). The water channel adjacent to Disk Island is named Lower Passage and has considerable boat traffic.

The beach segment used in the experimental testing was relatively flat with a very shallow slope. The distance from the toe to the top of the beach measured approximately 150 m, and the usable width measured approximately 100 m. It was bounded on one side by a large rock outcrop and on the other side by a field of boulders. The beach material was composed of cobbles of varying diameter (15 to 30 cm or more) atop a mixed sand and gravel base. The particle size distribution of a sample of underlying beach material was measured in the laboratory with the following ranges: 48.8% > 0.64 cm; 27.2% > 0.13 cm; 11.6% > 0.084 cm; 9.7% > 0.064 cm; 1.5% > 0.05 cm; and 1.1% > 0.04 cm. The beach was well protected from incoming storms and received little wave action unless the wind originated from due North. No history of oil deposition on this beach was known prior to the Exxon Valdez event. In September 1989, the beach segment still had relatively high amounts of oil remaining on it. During the summer months the water temperatures at Disk Island ranged between 13 and 16 °C, and surface

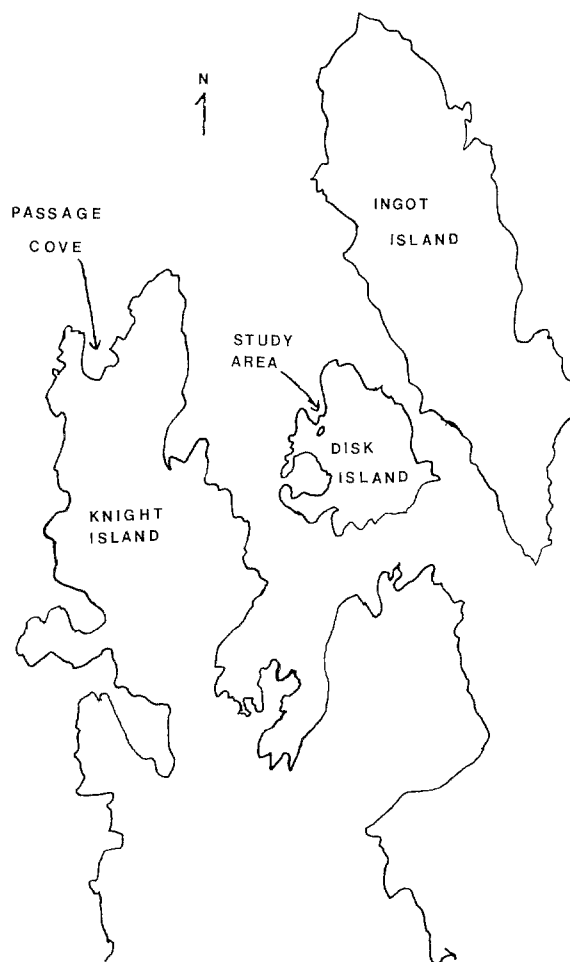


Fig. 1. The location of Disk Island relative to Knight and Ingot Islands in Prince William Sound, Alaska.

water salinities ranged between 20 and 22 ppt depending on rainfall.

Plot description

The experimental layout is depicted in Fig. 2. The experiment was a randomized complete block design. Four beach segments ('blocks'), each 20 m wide (labeled 1 through 4), were staked out in the intertidal zone approximately midway between the low and high tide lines. Within each block were 4 treatment plots, labeled A through D, 2 m wide by 5 m long (top-to-bottom). The plots were separated from each other by buffer zones measuring 3 m in width. Within each block plot A was the no-treatment control, plot B was the nutrient-only treatment, plot C received nutrients plus Sybron's product, and plot D received nutrients plus ERI's product. The treatment plots within each block were randomly distributed according to the following scheme: block 1,

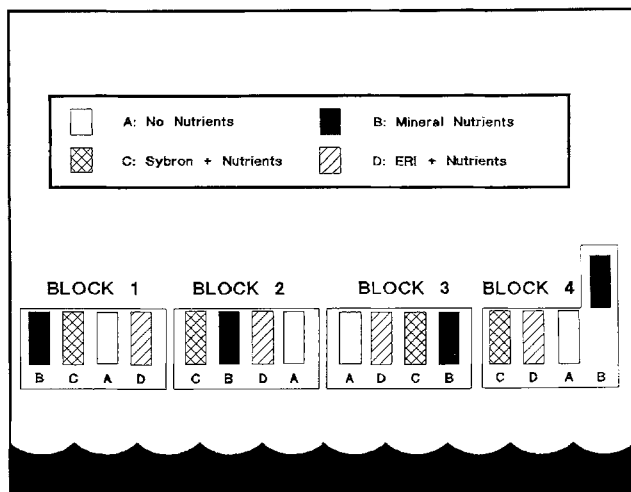


Fig. 2. The experimental plot layout on Disk Island.

BCAD; block 2, CBDA; block 3, ADCB; and block 4, CDAB. In block 4 the nutrient-treated plot was located above and to the right of the control plot (Fig. 2). This positioning was necessary because of the presence of compacted peat on the extreme right end of the beach. There was a surface flow of water from a saltwater lagoon located approximately 50 m above the test area that flowed across the nutrient-treated plot onto the control plot. This stream was not noticed when the plots were first laid out.

Each of the 16 plots was subdivided horizontally into three equal segments 2 m wide by 1.67 m long, as shown in Fig. 3. In each of the three segments, four bags, each made of fiberglass screening material and containing

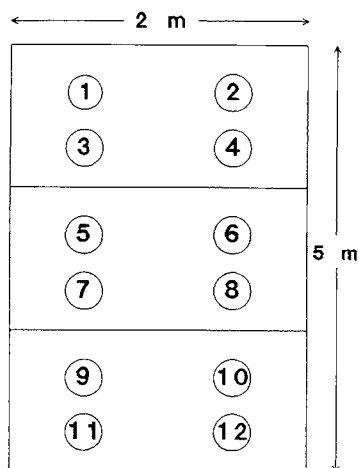


Fig. 3. Diagram of a typical beach plot showing dimensions and locations of sampling bags.

approximately 750 to 1000 g of uniformly sized oily gravel obtained from the high intertidal zone of the test beach, were buried approximately 5 cm below the surface and covered with mixed sand and gravel. The four bags corresponded to the four sampling events that were planned for the experiment. A surveyor's ribbon was attached to each bag for easy identification. The 12 samples within each block were numbered 1 through 4 in the top third, 5 through 8 in the middle third, and 9 through 12 in the bottom third.

The bags had been filled with gravel that had been sieved through a 25-mm coarse screen to remove large stones and then through a 4.75-mm sieve to remove the small sand granules that compact the beach material. The gravel was mixed manually by shovels and hoes in a large wooden container to achieve reasonable homogeneity in oil contamination and rock size. These bags served as samples to be taken on the appropriate sampling days.

Sampling

On a given sampling day, triplicate samples from each plot within a block were collected according to a random schedule. One sample was randomly taken from each of the three identical sectors of each plot. Some of the gravel was poured into 500-ml I-Chem jars, labelled, and placed in a cooler to be carried back to Valdez for freezing and shipment via Federal Express to the analytical chemistry laboratory located in Pittsburgh, PA. The rest of the gravel was archived in aluminum foil and frozen. Thus, 48 samples were collected on each of the four sampling days, giving a total of 192 samples for the entire experiment.

Sediment chemistry

Quantification of the aliphatic hydrocarbon target analytes was performed by gas chromatography using a flame ionization detector (GC/FID). PAH determinations were not performed. Chemical analysis of sediment extracts followed the same procedure as previously described [17]. Oil residue weights were determined by first extracting the oil from 100 g of the sediment sample with methylene chloride and evaporating to dryness. Resultant dry residues were weighed on an analytical balance and recorded. Residues were then redissolved in methylene chloride, fractionated on a silica gel column, and gas chromatographic analysis conducted as described [17].

Microbiology

Subsamples from the eight plots of blocks 2 and 3 were analyzed in duplicate for oil degrading bacteria by standard plate count, using Bushnell-Haas medium [4] supplemented with Alaska North Slope crude oil as the carbon source. Only one of the three triplicate bag samples from each of the eight plots was analyzed for microbial num-

bers. The plates were incubated at 15 °C for 21 days and the colonies counted.

Nutrients

To track the fate of the added nutrients with time, wells were installed in some of the plots for collecting subsurface water samples. Well points measuring 5 cm I.D. by 90 cm long were driven into the center of the four control plots and the four plots which received only nutrients. Subsurface water from these eight wells, which served as samples for nutrient analysis, were collected according to a pre-determined sampling schedule (see below).

Nutrient sampling

Samples of water for nutrient analysis were collected by pumping from a battery-operated peristaltic pump connected to silicone rubber tubing placed in the bore of the well. The pump was operated for approximately 1 min, to clear the tubing and well of carry-over water. Approximately 500 ml water was collected in clean, amber, polyethylene bottles and returned to the laboratory for analysis. The samples were filtered through a glass fiber filter prior to chemical analysis.

Application of nutrients

The source of nitrogen was ammonium nitrate. Each 2 m × 5 m plot received 200 g of N (20 g/m²). At 35% N, the amount of NH₄NO₃ containing 200 g of N was 570 g or 1.25 lb per plot. This amount, less approximately 40 g to account for the N in the product containing the phosphate salt (see next paragraph), was added to 6 gallons of seawater and the contents were stirred until the salts dissolved. A 2-gallon plastic sprinkling can was filled with the solution and the entire contents were poured onto the top third of a plot earmarked for nutrients. The sprinkling can was again filled and the contents poured onto the middle third. The procedure was repeated for the bottom third.

The source of phosphorus was an Ortho product named 'Upstart', which had an N-P-K content of 3-10-3. At 10% P₂O₅, the amount of Upstart used was 450 g (1 lb) per plot. This corresponded to a phosphorus loading of 20 g P per plot (2 g P/m²). The 450 g of Upstart was added to the 6 gallons of seawater above, after the NH₄NO₃ had been dissolved and before applying fertilizer solution to each plot. This product contained 3% N in the form of NH₄NO₃. The amount of N in Upstart had already been accounted for in the above 530-g computation of NH₄NO₃ needs.

Nutrient analysis

The nitrogen species NH₃-N, NO₂⁻-N, and NO₃⁻-N were determined by U.S. EPA Methods [16]. The NH₃-N method was No. 350.1 and the NO₂-N/NO₃-N method was No. 353.1.

Schedule

The entire experiment lasted only 27 days because severe Alaskan winter weather precluded field activities beyond the month of August. Day 0 was Sunday, July 29, 1990. Nutrients and commercial products were applied on days 0, 4, 8, 12, 16, 20, and 24. One extra application day, day 2, was used for an additional commercial product application, as specified by the two vendors. After nutrients and products had been delivered to the appropriate plots, randomly assigned triplicate sampling bags were removed from the plots for time 0 sediment chemistry and microbiology analysis. The other triplicate sampling bags were collected on days 9, 18, and 27. Nutrient sampling took place on days 1, 2, 3, 4, 17, 18, 19, and 20. This allowed determination of nutrient concentrations throughout the four-day interval between applications at two different times in the experiment.

Data analysis

The data were analyzed by analysis of variance using SAS Software Release 6.06 on an IBM Model 3090 mainframe computer.

RESULTS

Persistence of nutrients

Figures 4–6 summarize the average changes in nutrient levels with time in each block.

Persistence of ammonia-N was the most erratic (Fig. 4). In block 1 the levels of NH₃-N in the nutrient-

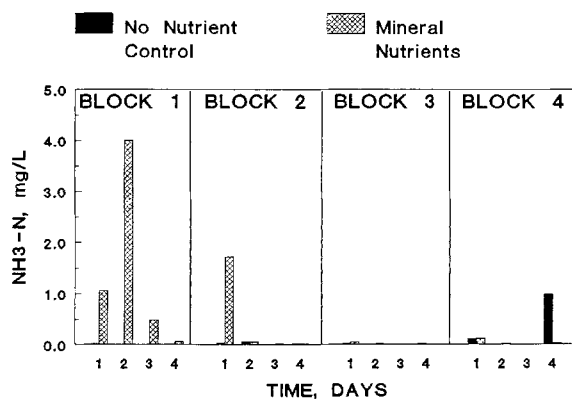


Fig. 4. Average changes in ammonia-N levels in the four days between fertilizer applications.

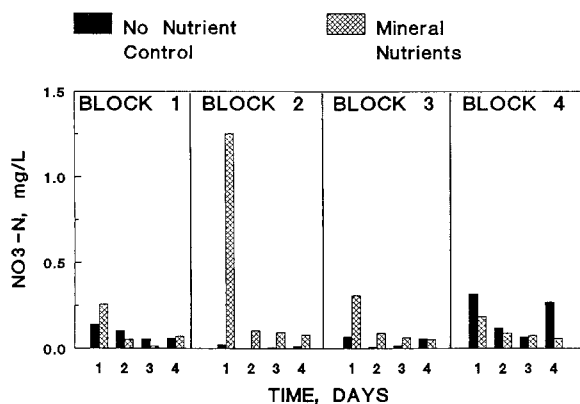


Fig. 5. Average changes in nitrate-N levels in the four days between fertilizer applications.

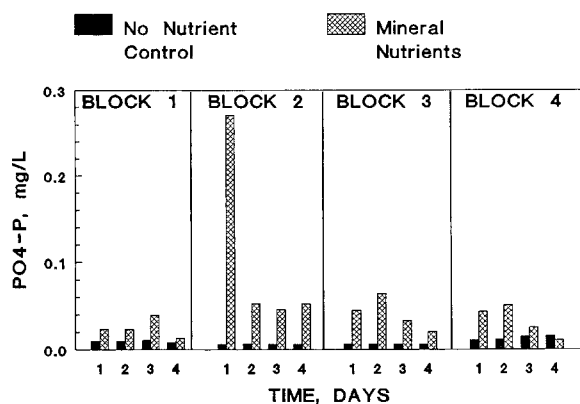


Fig. 6. Average changes in phosphorus levels in the four days between fertilizer applications.

treated plot were measured at 1.1 and 4.0 mg/l one and two days after application, respectively, and in block 2 the $\text{NH}_3\text{-N}$ was 1.7 mg/l one day after application. Little $\text{NH}_3\text{-N}$ was measured in any of the control plots at any time except in block 4, where 0.1 mg/l was measured after one day and almost 1.0 mg/l after four days.

The nitrate and phosphate data indicate significant but decreasing levels of nutrients in the nutrient-treated plots as time progressed to four days after application (Figs. 5 and 6). Again, high levels of $\text{NO}_3\text{-N}$ and measurable levels of $\text{PO}_4\text{-P}$ appeared in the control plot of the fourth block four days after application.

Changes in oil degraders

Oil degrader counts in all plots of blocks 2 and 3 are shown in Fig. 7. Although the levels of oil degraders were high in each of the plots, there were no significant changes or differences in any of the plots after 27 days of field testing.

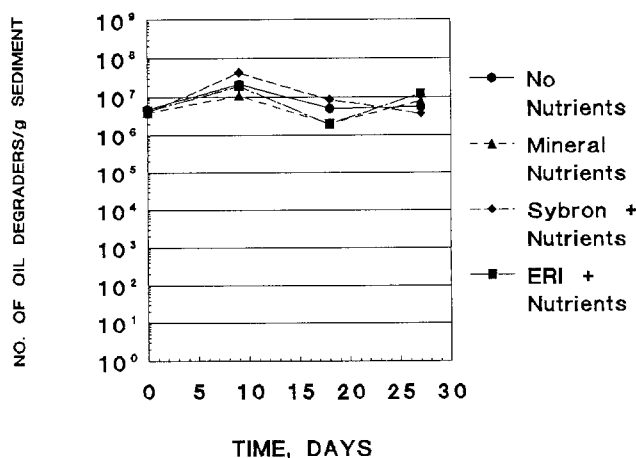


Fig. 7. Oil degrader counts in all plots of blocks 2 and 3 as a function of time.

Oil residue weight

Changes in oil residue weight, averaged over all four blocks, are summarized in Fig. 8 as a function of time. The points on the connected curves are the mean residue weights for each of the four treatments. The error bars depict one standard deviation unit above and below the means. Error bars represent the variation in oil residue weight among the four blocks and are indicative of the overall experimental error.

Visual inspection of the data from the plots treated with mineral nutrients alone and mineral nutrients supplemented with Sybron's product indicates a decrease in oil residue weight of approximately 33% at the end of the experimental period, compared to no net change in the no-nutrient control plot and a slight increase in the ERI plot. When the data were subjected to analysis of variance, however, there were no statistically significant differences among any of the four treatments at the 5% significance level.

The broad error bars on Fig. 8 at the day 0 sampling time are compared to the other three sampling times. Despite the effort to control the heterogeneity of rock size and contamination by the sieving and mixing techniques prior to start-up, there was still substantial variation in oil residue weight from plot to plot and block to block at day 0. To ascertain the source of this variation, a breakout of plot oil residue weights by block was conducted. Results are shown in Fig. 9. Examination of these data reveals the differences in the distribution of oil from plot to plot. Very little change took place in any of the treatments in blocks 2 and 4. Oil residue weights in the no-nutrient control plot and the nutrient-only plot of block 1 and the Sybron plot of block 2 declined markedly within the first nine days and then leveled off for the remainder of the experimental period. The oil residue weights in the ERI

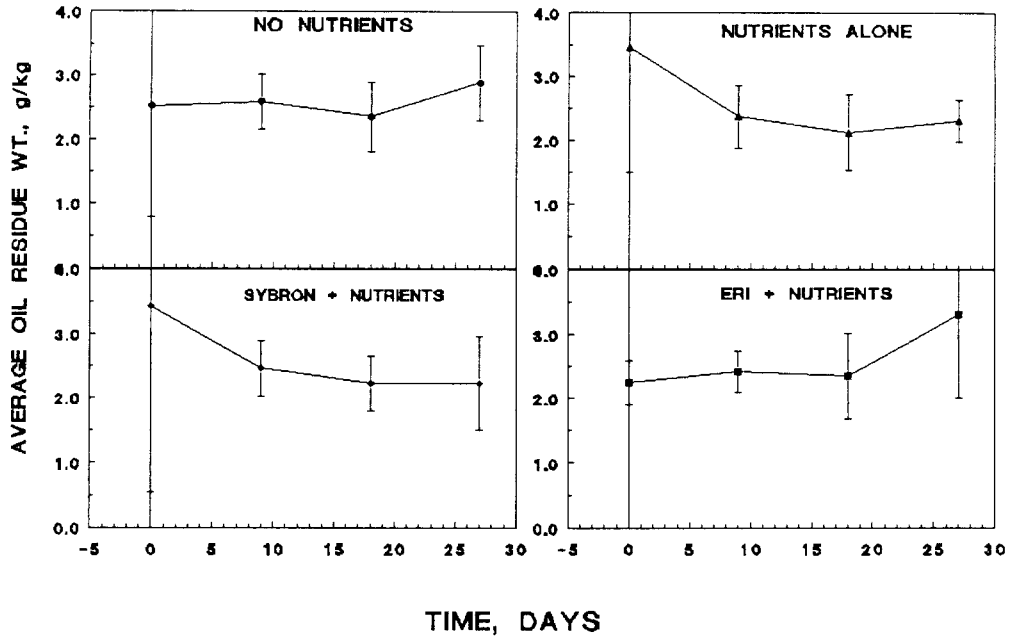


Fig. 8. Changes in oil residue weight averaged over all four blocks as a function of time. Error bars indicate one standard deviation.

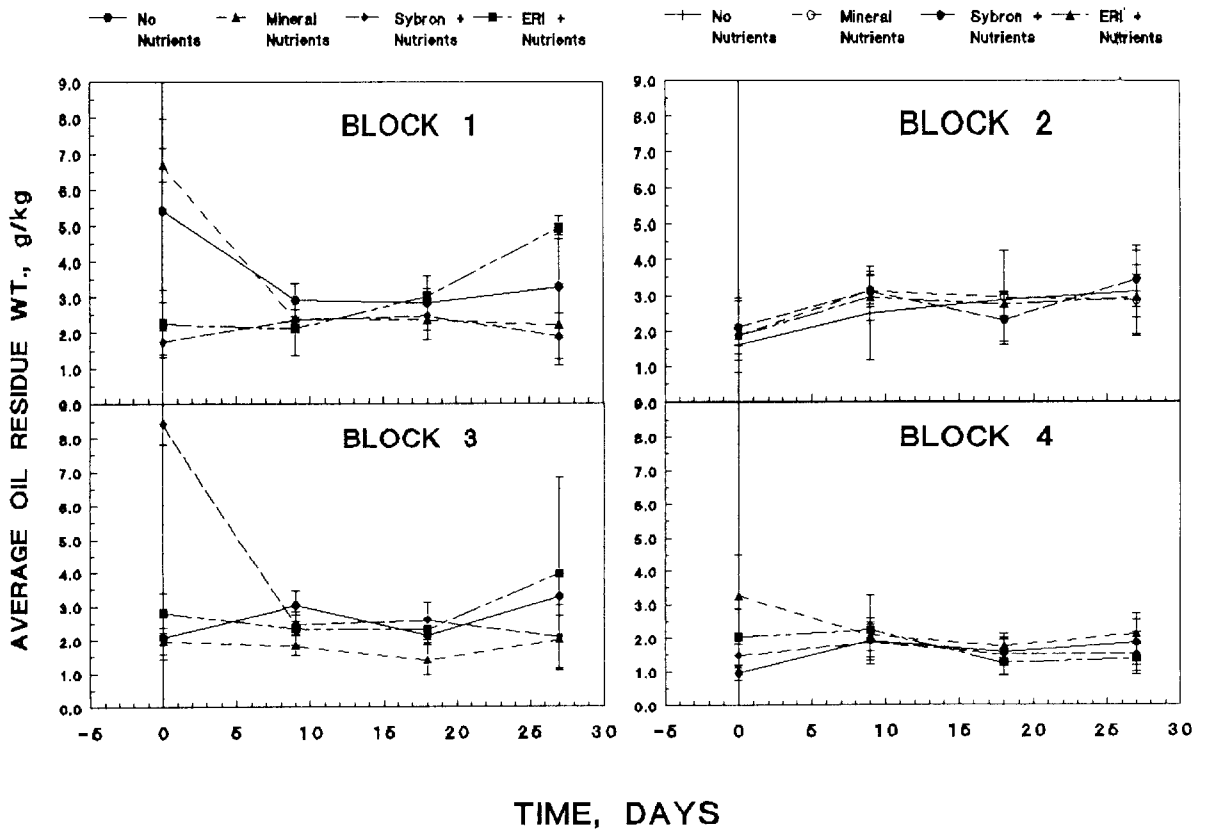


Fig. 9. Changes in oil residue weight in each block as a function of time. Error bars indicate one standard deviation.

plots of blocks 1 and 3 showed an increase between days 18 and 27.

The error bars shown in Fig. 9 are indicative of the sampling error of triplicate samples. At day 0 the agreement of the triplicate samples averaged within each plot (the within-plot variation, Fig. 9) is better than the agreement of identical plots averaged over blocks (the between-plot variation, Fig. 8). This suggests that the cause of the variation among plots was consistent within each of the plots.

Total resolvable alkanes

All samples were subjected to GC analysis to determine the changes in the aliphatic profiles of the oil among the various treatments. The concentrations of all the normal alkanes and the isoprenoid alkanes pristane and phytane resolvable by GC/FID were summed together for each treatment, averaged over all four blocks, and plotted as a function of time. The data with associated error bars are shown in Fig. 10.

Except for the day 0 data, the error bars in Fig. 10 are generally higher than the corresponding residue weight error bars (Fig. 8). Although a downward trend in resolvable alkane concentrations is perceptible in all of the treatments after 27 days, the analysis of variance revealed no significant differences among the treatments ($P < 0.05$). This agrees with the findings of no significance among treatments in the oil residue weight data.

Figure 11 was constructed to examine the behavior of

the GC data in the individual plots within each block. A general, downward trend in alkane hydrocarbon levels is perceptible in the control, nutrient-only, and Sybron-plots of blocks 1 to 3 and the ERI-plot of block 1. Temporal changes in the alkane levels from the ERI-plot of block 2 are highly variable, showing an increase at day 9 followed by decreases at days 18 and 27, while in block 3 increases are observed successively after day 9.

The error bars represent the sampling error associated with the triplicate samples in each plot. The error bars are higher overall than the corresponding oil residue weight data (Fig. 9). The sampling errors associated with the GC data appear to be no better than the overall experimental error, which contrasts somewhat with the residue weight data.

Total resolvable alkanes as a percent of the residue weight

The previous two figures depicted observed temporal changes in total resolvable alkanes normalized to sediment weight but not to the weight of the oil. Since there might have been significant differences in the extent of sediment contamination among samples, the measured resolvable alkanes were normalized to oil residue weight within each sample; the results are shown in Figs. 12 and 13. Figure 12 shows changes in the total resolvable alkanes as a percent of the residue weight, averaged over all four blocks for each treatment, and plotted as a function of time. Figure 13 shows the changes within each block. Again, there were no significant differences among

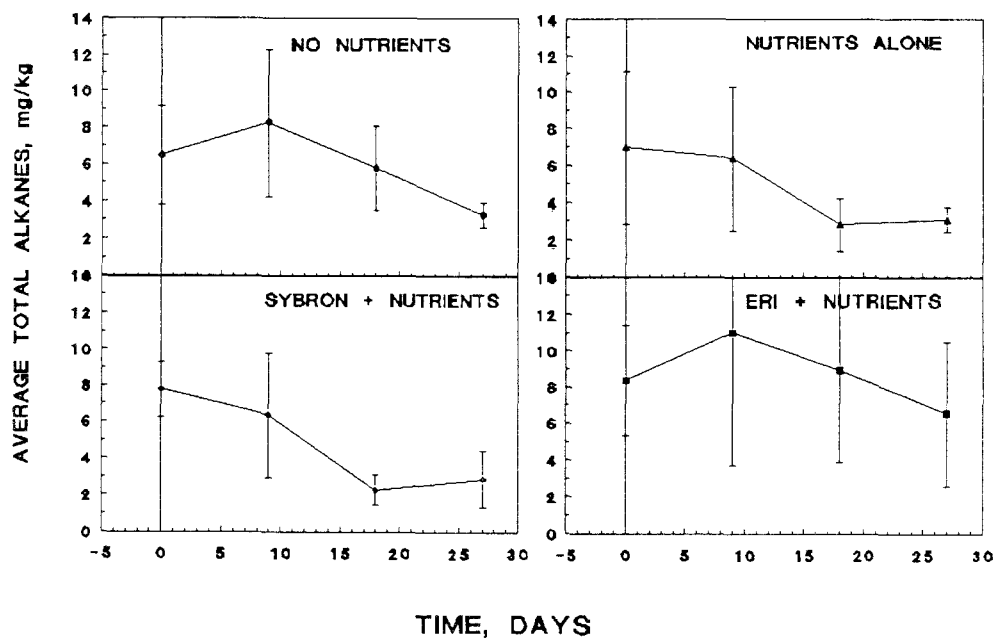


Fig. 10. Changes in total resolvable alkanes averaged over all four blocks as a function of time. Error bars indicate one standard deviation.

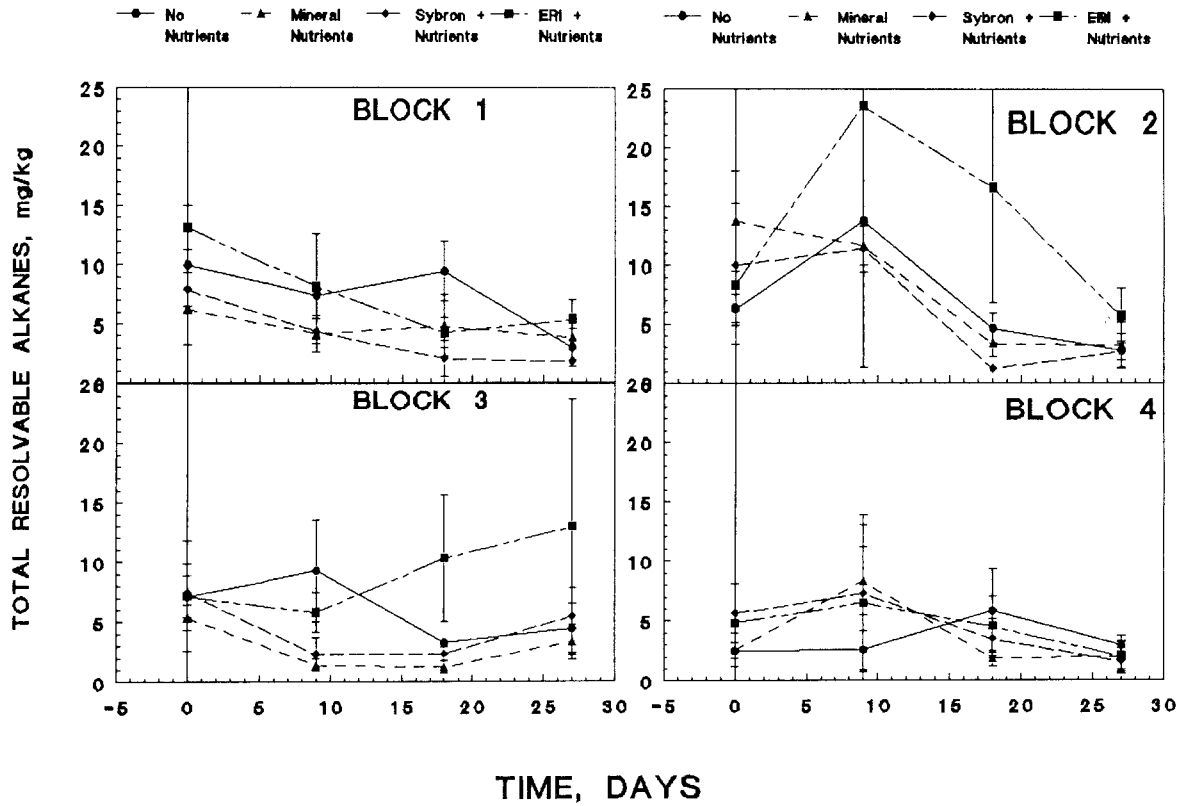


Fig. 11. Changes in total resolvable alkanes in each block as a function of time. Error bars indicate one standard deviation.

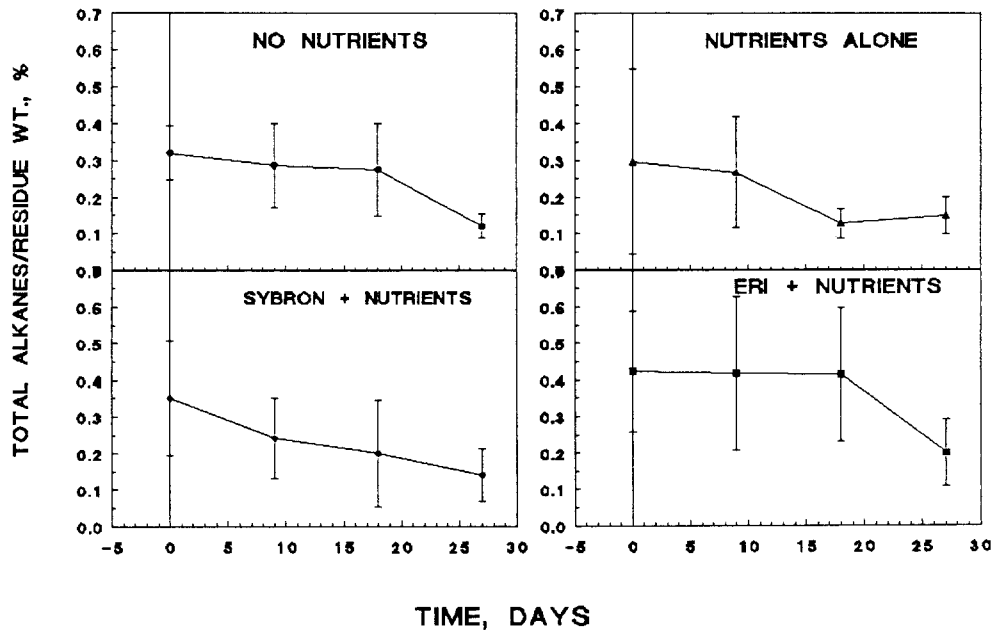


Fig. 12. Changes in total resolvable alkanes as a percent of oil residue weight averaged over all four blocks as a function of time. Error bars indicate one standard deviation.

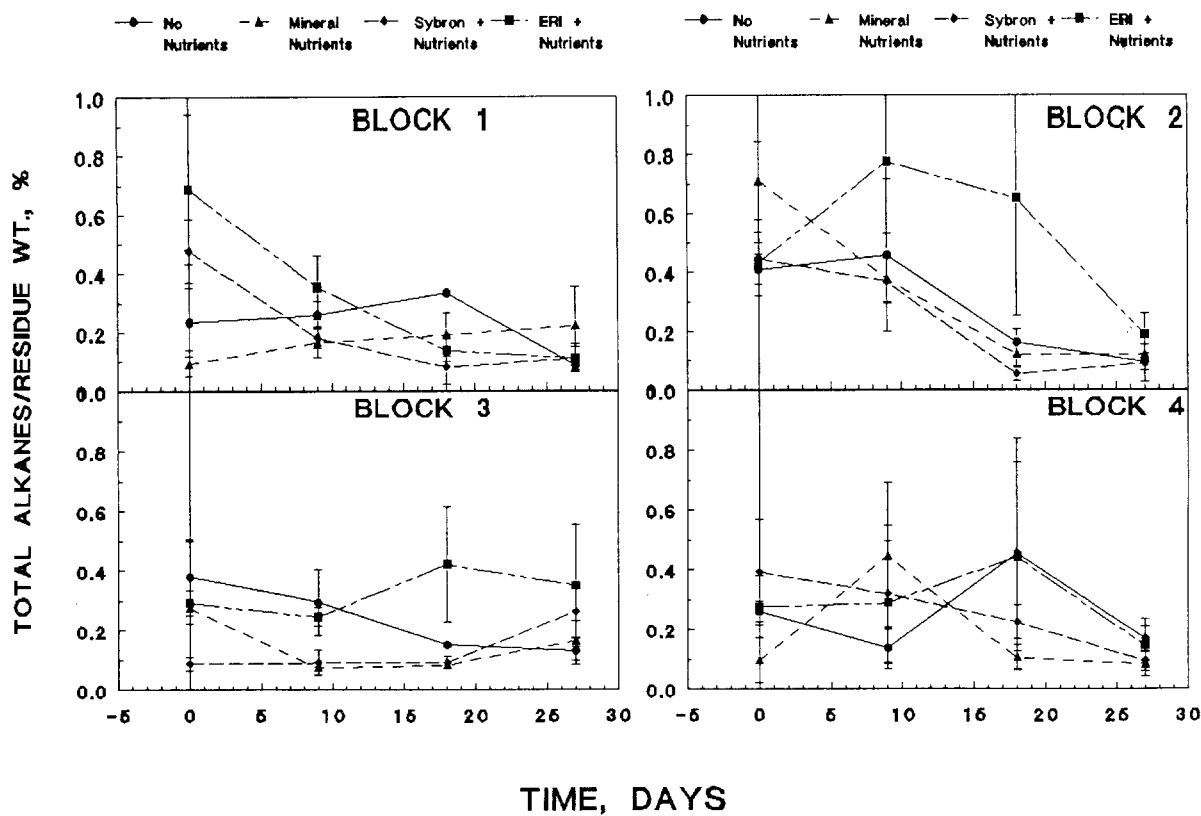


Fig. 13. Changes in total resolvable alkanes as a percent of oil residue weight in each block as a function of time. Error bars indicate one standard deviation.

the four treatments. Note that the general behavior of the residue weight-normalized curves in Figs. 12 and 13 is similar to the corresponding behaviour of the sediment weight-normalized curves in Figs. 10 and 11. This suggests that contamination of sediment samples was homogeneous.

One important observation from Fig. 12 is the magnitude of the total alkane/residue weight ratio. The total alkane hydrocarbons resolvable by GC/FID are less than 0.5% of the total oil residue weight. In other words, over 99.5% of the oil remaining on Disk Island 1.5 years after the spill is not resolvable by conventional gas chromatography. The compounds comprising this persistent fraction are likely the tars and asphaltenes that degrade slowly with time.

DISCUSSION

The conclusions reached in this field study were based on three sources of information: nutrient persistence, microbiology, and sediment chemistry. Nitrogen and phosphorus clearly persisted at measurably higher levels in the treated plots compared to the control plots throughout the four days between applications. These

measurements were taken approximately 60 to 90 cm below the surface of the beach, suggesting that nutrients were in constant contact with the subsurface sediment layers for relatively long periods of time.

Well samples were collected while the tide was low. The water in the well bores could only have come from the beach subsurface. Salinity and dissolved oxygen measurements taken at the site indicate that water in the wells was a mixture of fresh and salt water. From this information we conclude that the bag enclosures did come in contact with the added nutrients.

The source of the high $\text{NH}_3\text{-N}$ spike in the control plot of the fourth block may have been caused by carry-over of nutrients from the nutrient-treated plot onto the control plot (Fig. 2). Although this explains the higher levels of $\text{NH}_3\text{-N}$ measured one day after application, it does not explain why a high spike was observed on the fourth day.

The microbiology data clearly demonstrate no net increase in oil degrader populations in any of the plots after 27 days and no differences among the four treatments at any time during the 27-day period. The oil degrader populations were high to begin with and were maintained with or without the presence of excess nutrients. Either the oil degraders were dormant or, more

likely, they were sufficiently able to sustain their activity with the oligotrophic levels of nutrients present in the ambient environment.

Oil agar plates have been used satisfactorily for estimating hydrocarbon-utilizing microorganisms [1,8,9,14, 15]. Although it can be argued that oligotrophic bacteria could grow on the oil agar plates, prior experience in our laboratory with Prince William Sound samples has shown that oil-degrading organisms are the predominant group. Oligotrophs are frequently suppressed by the high concentrations of carbon in the medium. Even if they had been present, the error would have been consistent for all samples, because each sample would have had the same background population. For the purposes of this study, an estimate of the populations was all that was required. Statistical comparison of the treatment plots was based only on oil chemical analysis, not microbiology.

Sediment chemistry provided the basis for the statistical analyses conducted. No significant differences were found among the four treatments at the 5% significance level either from the standpoint of oil residue weight, total resolvable alkane hydrocarbons, or total resolvable alkanes as a percent of oil residue weight. The high variation observed in the data points out the necessity not only to replicate treatments when conducting field experiments but also to collect sufficient samples to provide statistical validity to the conclusions.

Examination of the computed errors at day 27 for the total resolvable alkanes revealed that the source of variation in the data was equally divided among treatments, blocks, and overall experimental error. In other words, one-third of the observed variation was due to differences among treatments, one-third to differences among blocks, and one-third to the inherent variability in the experiment.

It can be argued that the high variability in oil analyses could have led to a Type II statistical error, i.e., failure to reject a false null hypothesis. To determine the probability of making such an error, we conducted a power analysis. The 'power' of a statistical test is the probability of rejecting the null hypothesis when it is false. The power of the test increases: (1) the greater the difference in population treatment means; or (2) the smaller the experimental error variance. Using the estimates of the treatment means and experimental error variance that resulted from the experiment, an estimate of the power of the experiment was derived. The probability of rejecting the null hypothesis was found to be 41% for total resolvable alkanes and 32% for residue weights. That is, if the true means differed by the same amount as the sample means, there is only a 41% chance of detecting such a difference from the alkane data and a 32% chance from the residue weight data. Thus, the experimental error variance was indeed too high to reliably detect differences of the type

observed in this experiment. The true differences among population treatment means would have to have been about 1.5-fold for the alkane data and 1.7-fold for the residue weight data for there to have been a 75% chance of correctly rejecting the null hypothesis. Considering the actual difference in population means observed, the reduction of the error variance would have to be about 54% for the alkane data and 66% for the residue weight data to have resulted in a 75% chance of correctly rejecting the null hypothesis. The only way to have achieved this result was to have taken more samples or set up more blocks. This power analysis emphasizes the fact that it cannot be concluded from this experiment that there was no difference in treatments. We can only say that there was insufficient evidence that the treatments differed.

Most of the readily biodegradable compounds in the aliphatic fraction of the contaminating oil had disappeared in the 1.5 years since the spill took place off Bligh Reef in Prince William Sound. This is one likely explanation for the lack of any significant enhancement by either nutrient addition alone or nutrient addition supplemented with commercial microbial cultures. Further evidence supporting this conclusion derives from examining the *n*-alkane/isoprenoid alkane ratios. These ratios have been used in past literature to indicate extent of biodegradation; the lower the ratio, the more extensive the biodegradation. The average *n*-C17/pristane and *n*-C18/phytane ratios on day 0 for all the plots on Disk Island were 0.18 and 0.27, respectively. This compares to approximately 1.5 to 1.8 for unweathered Alaska North Slope crude oil. Thus, the remaining oil present on Disk Island will likely degrade very slowly from now on because of the recalcitrant nature of the substrate. If either nutrient application or commercial inoculation can accelerate this rate, the time period must extend significantly beyond the 27 days allotted for this study or the trial must be conducted on beaches with fresher oil contamination.

There is ample evidence in the literature that supports the negative or inconclusive findings above. Rashid [13], studying oil degradation on the shoreline of Nova Scotia 3.5 years after the tanker Arrow ran aground in 1970, found that degradation was greatest in high wave energy environments and lowest in protected embayments. Disk Island is typical of a protected embayment. Colwell et al. [6] found that biodegradation of petroleum spilled from the tanker Metula in the Straits of Magellan in 1974 proceeded relatively slowly with marked persistence of oil two years after the spill. They attributed the slow rate of oil decline to the low concentrations of nitrogen and phosphorus available in seawater as well as restricted accessibility to degradable compounds within aggregated oil or tar balls. Microbial degradation was not effective in

attacking buried oil or oil that had formed asphalt layers on beaches. The latter observation is similar to the conditions existing on Disk Island in the current study. Others [2,3,18], upon studying the degradation of oil from the Amoco Cadiz spill, concluded that oil that was buried, oil within anoxic sediments, and oil within protected embayments appears to be the most persistent. Conditions that enhance aeration and resupply nutrients, such as high-energy wave action, favor biodegradation. Such conditions do not exist at Disk Island.

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