Journal of Industrial Microbiology & Biotechnology (2001) 27, 72–79

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Microbial community dynamics and evaluation of bioremediation strategies in oil-impacted salt marsh sediment microcosms

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Microbial community dynamics in wetlands microcosms emended with commercial products (surfactant, a biological agent, and nutrients) designed to enhance bioremediation was followed for 3 months. The effectiveness of enhanced degradation was assessed by determining residual concentrations of individual petroleum hydrocarbons by GC/MS. The size and composition of the sediment microbial community was assessed using a variety of indices, including bacterial plate counts, MPNs, and DNA hybridizations with domain- and group-specific oligonucleotide probes. The addition of inorganic nutrients was the most effective treatment for the enhancement of oil degradation, resulting in marked degradation of petroleum alkanes and a lesser extent of degradation of aromatic oil constituents. The enhanced degradation was associated with increases in the amount of extractable microbial DNA and *Streptomyces* in the sediment, although not with increased viable counts (plate counts, MPN). Bacteria introduced with one of the proprietary products were still detected in the microcosms after 3 months, but were not a major quantitative constituent of the community. The biological product enhanced oil degradation relative to the control, but to a lesser extent than the nutrient additions alone. In contrast, application of the surfactant to the oil-impacted sediment decreased oil degradation. *Journal of Industrial Microbiology & Biotechnology* (2001) **27,** 72–79.

Keywords: bioremediation; DNA hybridization; microbial community; oil contamination

Introduction

Oil extraction, refining, and transshipment activities are often located in coastal regions, putting wetland systems at risk for exposure to spilled oil. The inaccessibility of many sites and the fragile nature of wetlands ecosystems preclude mechanical cleanup of oil, making bioremediation a preferred option. Bioremediation strategies that have been proposed for oil spills in wetlands include fertilization, solubilization of oil, and bioaugmentation with oil-degrading bacteria [3]. Although bioaugmentation has been demonstrated to be effective in engineered systems [13], the ability of introduced organisms to establish themselves in the complex web of relationships among indigenous microbes is questionable. Moreover, supplies of oxygen and nutrients may be insufficient for concurrent degradation of oil and natural substrates. Using microcosm test systems, we evaluated the effectiveness of bioremediation strategies (fertilization, solubilization, and bioaugmentation) in light of the established sediment microbial ecology, with a focus on the dynamics of the bacterial populations.

Traditional methods (e.g., most-probable-number, MPN) have been used to monitor the response of microorganisms in oil-polluted systems [2,21]. However, culturable bacteria are not representative of natural bacterial communities [1]. A more comprehensive evaluation of bacterial communities from environmental samples can be achieved by application of molecular methods [1,16,17].

Phylogenetic probing is an inexpensive and rapid technique for detecting broad-scale shifts in microbial community structure [4,21,23]. In these experiments, the community structure is defined by quantifying the amount of broad-spectrum probes that hybridizes to nucleic acid extracted from the environmental samples.

This study used phylogenetically nested probes (16S rRNA) in hybridization assays to monitor changes in the microbial communities of petroleum hydrocarbon (PHC)-contaminated sediment amended with bioremediation products. Sediment-extracted DNA was hybridized with domain probes for Archaea, Bacteria, and Eukarya, and representatives of oil-degrading bacterial groups including *Pseudomonas*, *Acinetobacter*, and *Streptomyces* [2,28]. Traditional techniques (total MPN and MPN oil degraders) were used to quantify numbers of functionally significant bacteria [24]. These analyses were integrated with gas chromatographic (GC/MS) measurements of PHC concentration for evaluating the effectiveness of the bioremediation products.

Materials and methods

Microcosm design and operation

Sediment microcosms were constructed of glass columns (10 cm i.D. × 20 cm) fitted with fritted glass supports (Figure 1). Plexiglass plates, drilled for pumping operations and drainage, were fitted to the tops and bottoms of the columns with Viton gaskets. Viton tubing (0.32 cm o.d.) was used for all applications that involved contact with effluent water. Tygon tubing was used in applications that involved contact with influent water only. The effluent was drained by pumping from a glass tube (0.32 cm o.d.) that extended to 1.5 mm above the sediment surface within a weir constructed from a cross-sectional cut of glass tubing (2.5 cm i.d.) and filled with sand.

The columns were operated in banks, with influent and effluent flows controlled by peristaltic pumps attached to timers. Filling and draining (5 cm depth, 390 ml volume) occurred over 3-h periods.

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Received 18 March 2001; accepted 9 June 2001

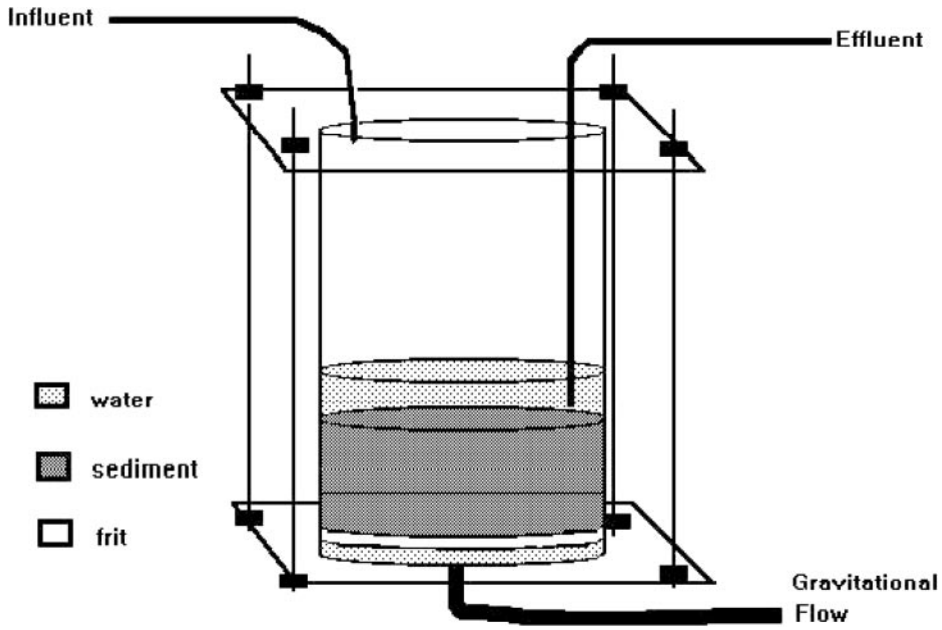

Figure 1 Diagram of a sediment microcosm with inflow and outflow controlled by a peristaltic pump.

The microcosms were left filled and drained for 3 h each, mimicking a 12-h tidal period.

Sediments were collected from Jack's Creek (Sapelo Island, GA) as grab samples and were transported to the laboratory on ice. In the laboratory, the fine, high-organic sediments were sieved through a 2.0-mm sieve to remove debris and refrigerated at 4°C. To obtain homogeneous columns and to increase reproducibility among replicates, sediments were added to 12 columns as slurries. Columns were allowed to settle by gravity (final depth 5 cm) and were incubated at room temperature for 24 h to reestablish an anaerobic subsurface zone prior to the addition of oil.

The 521 fraction (the fraction remaining after distillation at 374°C, 20 mm Hg, the atmospheric equivalent of 521°C; ISO 1989) of Alaska North Slope (ANS) crude (Exxon, Houston, TX) was adopted as the test oil based on its chemical similarity to the slightly weathered oil that contaminated shorelines during the Exxon Valdez incident. The oil was added to the microcosms after settling of the sediment and re-establishment of the anaerobic–aerobic zones.

Test products and media

The salinity of the treatment systems was matched to that of the collection site (20% or 1.013 specific gravity at 21°C), which is characteristic of coastal marshes. Thus, artificial sea water (Instant Ocean, Aquarium Systems, Mentor, OH) was diluted with distilled water to the desired salinity.

A 0.1-N strength mineral salts medium, containing (mg) 20 $MgSO_4 \cdot 7H_2O$, 50 NH_4NO_3, 80 K_2HPO_4, 20 KH_2PO_4, 0.5 $CaCl_2 \cdot 2H_2O$, and 0.2 $FeCl_3 \cdot 6H_2O$ per liter, was used as the nutrient treatment. Nutrient solutions were prepared from autoclaved stocks and aerated prior to use.

A surface oil thickness of 0.5 mm was created by adding 3.985 ml of the 521 fraction of the ANS crude oil to each microcosm. The microcosms were cycled for 24 h after addition of the oil and prior to addition of bioremediation products (treatments) to be tested.

Product application

Bioremediation products were submitted for anonymous testing through the National Environmental Technology Applications Committee by individual manufacturers. Products were accompanied by material safety data sheets and recommendations for their application under field and test conditions.

Product E was identified as a biological product. It consisted of three components: a suspension of nonpathogenic bacteria in a bran carrier, a bacterial activator solution, and a nutrient mixture. A mixture (1.6 ml) of the three constituents was applied weekly at a rate based on the amount of oil in the test microcosms, yielding 18.2 mg nitrogen and 1.8 mg phosphorus per application.

Product I was identified as a mixture of ethoxylated linear alcohol, polyethylene sorbitan monolaurate, alcohol derivatives, and a proprietary blend of anionic surfactants. A 10:1 dilution of this product was applied at a 5:1 ratio (v/v) of product:oil on day 0 and was reapplied at lower dilutions on days 5 (2:1) and 10 (1:1).

Product N was not a submitted test product, but rather was a nutrient solution based on a 0.1 N solution of the mineral salts mixture described above. Nutrients were added to the artificial sea water that was exchanged tidally as described above, resulting in an application of 0.75 mg P and 1.4 mg N per tidal cycle per microcosm, or twice that daily.

On a weekly basis then, microcosms dosed with Product E received 18.2 mg N and 1.8 mg P in a one-time dose, and those dosed with Product N received 13.6 mg N and 10.5 mg P per week.

Analyses of microcosms

Oil extraction and chemical analysis of PHC: Each sediment core was sacrificed and divided into three layers: two layers of 2 cm each and a remaining bottom layer. Each layer was carefully homogenized with a spatula and transferred to Teflon centrifuge tubes. The sediments were extracted with two 125-ml methanol additions and three 100-ml methylene chloride

additions. After each solvent addition, the sample was agitated for 2 h with a wrist action shaker (Lab-Line Instruments, Chicago, IL), and centrifuged for 5 min at 3000 rpm. All the supernatants were pooled in a 1000-ml separatory funnel to which 20% NaCl (200 ml) was added. The organic phase was collected and evaporated to 200 ml using an S-EVAP Analytical Evaporator (Organomation, MA).

In preparation for analysis, triplicate subsamples from layer 1 (20 ml each) were transferred to glass conical tubes. A recovery standard of acenaphthalene-d_{10} and chrysene-d_{12} (100 μg/ml each) (1 ml) was added to each sample and the volume was adjusted to 20 ml. Hexane (20 ml) was added and the sample was evaporated to one half its volume under a N_2 stream in a water bath at 38°C, effecting a solvent exchange and reducing the volume to 5 ml. The extracts were cleaned by passage through an activated silica column (63–200 mesh size) conditioned with hexane. The silica columns were prepared fresh each day by adding 10 ml of a silica–hexane slurry to 10 cm^3 glass syringes tapped with xylanized glass wool. After addition of the sample, the column was rinsed once with 10 ml methanol and three times with 10 ml methylene chloride. The sample was evaporated to approximately 2 ml under a N_2 stream in a water bath at 38°C, 1 ml internal standard (naphthalene-d_8 and phenanthrene-d_{10}, 100 μg/ml each) was added, and the final volume was adjusted to 5 ml. Samples were stored at 4°C until analysis.

Samples were analyzed on a Hewlett Packard 5972 mass spectrometric detector combined with a 5890 Series II gas chromatograph (GC/MS) using a split–splitless injector in the splitless mode and helium as the carrier gas [4]. C2 chrysene was used as an internal standard, and was utilized to normalize the data against a compound that is very resistant to degradation. Data were analyzed by analysis of variance using the StatView 4.1 program. A P value of <0.05 was considered necessary to establish a statistically significant difference between control and treated microcosms.

Microbiological analyses

Bacterial enumeration: The numbers of total bacteria and hydrocarbon-degrading bacteria were determined in triplicate samples of microcosm sediments prior to initiation of experiments and after 3 months of microcosm operation. Total bacteria were enumerated as colony-forming units (cfu) on 0.1 trypticase–soy agar plates incubated at room temperature (25°C). The numbers of hydrocarbon-degrading bacteria were determined using the sheen screen MPN technique [4–6].

Nucleic acid analysis

DNA extraction from sediments: DNA was extracted from sediments using a modified version of the procedure of Bachoon *et al* [4]. In short, the sediment (1 g wet weight) pellet was washed with 2 ml of 0.12 M sodium phosphate buffer, resuspended in 2 ml of lysozyme solution (15 mg/ml), and incubated with agitation (150 rpm) for 2 h at 37°C prior to the addition of 2 ml of a solution of 0.1 M NaCl, 0.1 M Na–EDTA (pH 8.0)–10% sodium dodecyl sulfate.

Cells were lysed by three cycles of freezing (−70°C for 30 min) and thawing (65°C for 5 min). The efficiency of cell lysis was determined by acridine orange direct cell counts (AODC) [11] with fluorescence microscopy (model BH-2; Olympus, Tokyo, Japan), and was greater than 95%. After the freeze–thaw cycles, the DNA was extracted with phenol:chloroform and precipitated with cold isopropanol.

The reddish brown nucleic acid pellet was purified with polyvinylpolypyrrolidine to remove humic material [27]. The purity of the extracted DNA was determined by electrophoresis in 0.8% agarose with or without digestion with the restriction enzymes *Sau*3AI and *DNase*1 [9]. DNA was quantified by fluorescence measurements using Hoescht 33258 dye (Sigma, St. Louis, MO) immediately before use [18].

Probe labeling: The oligonucleotide probes (Table 1) were synthesized using a Beckman DNA synthesizer (Oligo 1000) and following the manufacturer's protocol (Beckman, Fullerton, CA). Digoxigenin (DIG)-labeled probes were prepared with terminal transferase as described in the Genius 5 Oligonucleotide 3′-End Labeling Kit (Boehringer Mannheim, Indianapolis, IN).

Hybridization analysis: DNA samples were denatured by boiling them for 10 min and loaded on positively charged nylon membranes (0.45 mm pore size; Boehringer Mannheim) with a Manifold 1 dot-blot apparatus (Schleicher and Schuell, Keene, NH). The hybridization condition was optimized for each probe against genomic DNA from reference species of *Pseudomonas aeruginosa*, *Streptomyces viridosporus* TA7, *Acinetobacter calcoaceticus* BD4, and *Methanococcus voltae*. The membranes were UV-crosslinked (UV Stratalinka 2400; Stratagene) and prehybridized for 3 h in standard Genius hybridization buffer containing 10% formamide. Hybridization membranes were incubated overnight and washed in SSC buffer (0.3 M NaCl, 30 mM sodium citrate, pH 7.0) at the specified temperature for each probe (Table 1). Binding of the DIG-labeled probes was detected with the chemiluminescent substrate (CSPD, ready-to-

Table 1 Sequences, target sites, and hybridization conditions for oligonucleotide probes used in membrane hybridization

Probe	Sequence (5′ to 3′)	Target position	Temperature (°C)[a]	Reference
Universal	ACGGGCGGTTGTTA/GC	16S, 1392–1406	40	[21]
Eukarya	GGGCATCACAGACCTG	16S, 1209–1224	40	[13]
Bacteria	GCTGCCTCCCGTAGGAGT	16S, 338–355	45	[1]
Archaea	GTGCTCCCCCGCCAATTCCT	16S, 915–934	53	[10, 26]
Acinetobacter	ATCCTCTCCCATACTCTA	16S, 652–669	50	[29]
Streptomyces	GCGTCGAATTAAGCCACA	16S, 924–941	46	[25]
Pseudomonas (group 1)	GCTGGCCTACCCTTC	23S, 1432–1446	46	[22, 28]

[a]Empirically optimized for hybridization and membrane washes.

use; Boehringer Mannheim) and recorded on X-ray film with exposure times of 4–12 h. Under the optimized hybridization conditions (Table 1), the probes effectively discriminated between target and nontarget groups. Hybridization membranes

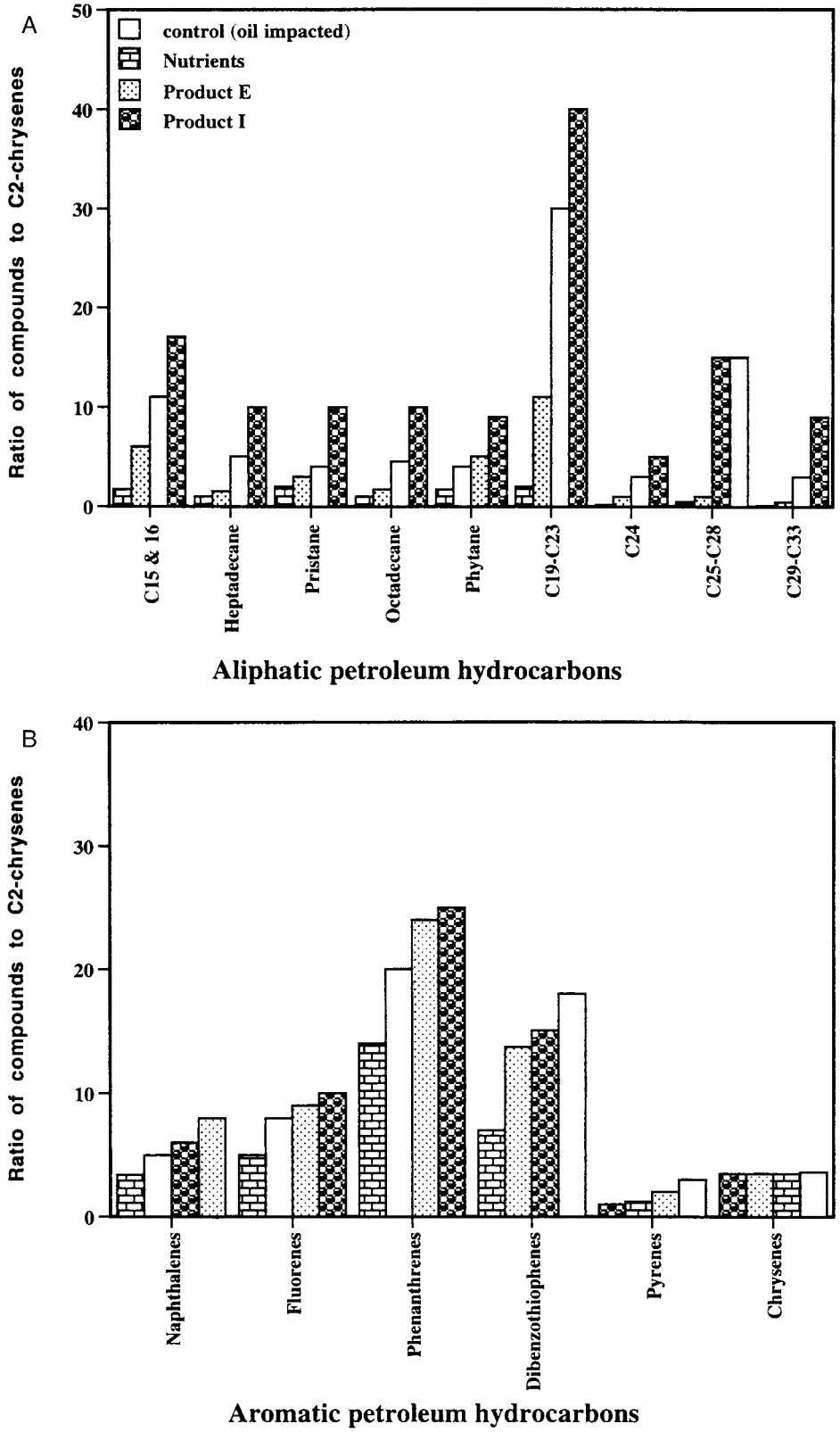

Figure 2 Quantities of aliphatic PHCs (A) and aromatic PHCs (B) remaining in oil-impacted sediment microcosms after 3 months, expressed as the concentration remaining normalized to the concentration of C2 chrysenes.

Table 2 Ratios of branched- and straight-chain alkenes in oil-impacted microcosms amended with bioremediation products after 3 months

	Control	Product E	Product I	Nutrients	521 Oil[a]
C17:pristane	1.03	0.40	1.03	0.28	1.58
C18:phytane	0.94	0.32	1.00	0.42	1.38

[a]Undegraded standard test oil (Alaska North Slope crude).

of sediment DNA extracts were prepared and hybridized as above, and included standard DNA (20–300 ng), sediment DNA extract from each microcosm treatment, and sediment DNA extract treated with DNase (negative control).

A linear regression of the quantity of standard DNA *versus* hybridization signal as determined with a laser densitometer (Molecular Dynamics, Sunnyvale, CA) was used to quantify the hybridization signal from the extracted DNA samples (r^2 between 0.950 and 1 in all cases). The Universal probe (1406R) served as a positive control. Hybridization signals with the Universal probe (not shown) were not significantly different between samples, confirming that the quantities of DNA spotted on the membrane were similar.

Product E analysis: The microbial component of Product E was characterized by AODC [11] and by hybridization analyses with the group-specific bacterial probes (as above).

Results

Degradation of hydrocarbons

Based on its conserved nature, 17*a*(*H*),21*b*(*H*)-hopane has been suggested as an internal marker for assessing the biodegradation of hybrocarbon components of crude oil [20]. In this study, hopanes were present in such low concentration that accurate quantification was problematic. Therefore, C2 chrysenes were sued as an internal marker rather than hopanes for assessing the PHC degradation [8].

Oil-impacted microcosms were used to evaluate bioremediation strategies in salt marsh sediment (Figure 1). Additions of nutrients and a biological product (Product E) to the oiled microcosms resulted in greater degradation of aliphatic hydrocarbons after 3 months than in the oil-impacted control (Figure 2A). The differences in extent of degradation were most pronounced for the longer chain alkanes. The concentration of residual alkanes of greater than 19 carbons, normalized to residual C2 chrysenes, in the nutrient-amended treatment was reduced to less than 10% of the residual values in the control treatment. The compound that underwent the least degradation in the nutrient-amended microcosms was pristane, the branched C17 compound which was still degraded to 48% of its level in the control microcosms. Product E addition resulted in slightly less degradation than the nutrient amendment, although greater than in the control treatment (Figure 2A). The branched alkanes were the least effectively degraded, with 76% of pristane and phytane remaining relative to the controls. Additions of Product I, the surfactant, resulted in residual concentrations of aliphatic hydrocarbons ranging from 30% to more than double the values of the controls.

The ratios of C17:pristane and C18:phytane (Table 2) can be taken as an indication of the initial degradation of oil [19]. The ratios for the nutrient treatment and Product E are both drastically reduced ($P<0.05$) with respect to the ratios that characterize the weathered 521 oil. The ratios for the control treatment indicate a lesser extent of degradation, and those resulting from additions of Product I still less degradation, although all treatments resulted in significant ($P=0.05$) degradation of the alkanes in the original oil.

The addition of Product I reduced the concentrations of dibenzothiophenes and pyrenes relative to the control treatment (Figure 2B), although the values of residual naphthalenes, fluorenes, phenanthrenes, and chrysenes were not significantly ($P=0.05$) different. That pattern of aromatic degradation was similar with the addition of Product E. Only the addition of nutrients produced a significant ($P<0.05$) degradation of all classes of aromatic compounds relative to the oil-impacted control (Figure 2B).

Bacterial populations

The bacterial populations of the initial (without oil) sediment and in response to applied treatments were assessed for investigating the bases of the biodegradation responses. Of particular interest were the relative effectiveness of indigenous bacteria (unamended and amended with nutrients and a dispersant; Product I) compared to that of introduced bacteria (Product E) at oil degradation and the sustained viability of organisms added to the microcosms. Microscopic examination of Product E stained with acridine orange revealed a large population of bacteria (2.1×10^{10} cells/g) and a smaller number of yeast-like cells (1.8×10^{8} cells/g), and plate counts of heterotrophs (LB agar) indicated that Product E contained 7.0×10^{8} cfu/g.

Sediment bacterial numbers, determined as cfu on TSA agar, were within one log unit (6.59–7.59) for all treatments (Table 3). Fewer than 0.1% of the bacteria in the initial sediment and oil-impacted control, as indicated by the MPN technique, were oil degraders. The numbers of total bacteria and oil degraders in the microcosms amended with nutrients and Product I did not differ significantly from either the initial sediment or the control treatment; the proportion of oil degraders increased slightly to nearly 1%. The greatest increase in the proportion of oil degraders (10%) relative to cfu occurred in response to the addition of Product E, a biologic product.

DNA yield

DNA extractions from the sediment treatments were used to provide a culture-independent estimate of the microbial population densities [4,23]. DNA yields from the control microcosms (5.2 μg/g)

Table 3 Extracted DNA, bacterial enumeration by plate count (cfu), and oil-degrading bacteria (MPN oil degraders) in 1 g of the top oil-impacted marine sediment after 3 months

Microcosm	DNA (μg/g)	Log cfu	Log MPN (oil degraders)
Initial sediment	4.62±2.2	7.55±0.51	4.81±0.81
Control	5.21±0.6	7.38±2.23	4.70±1.20
Nutrient	8.71±1.1	7.45±0.52	5.11±1.13
Product E	8.32±1.2	7.38±0.23	6.01±2.01
Product I	5.90+2.3	7.41+0.50	4.80+0.10

Data show mean values±SD ($n=3$).
DNA was extracted from sediments taken from triplicate microcosms. Each initial (intact) sediment was obtained at time zero.

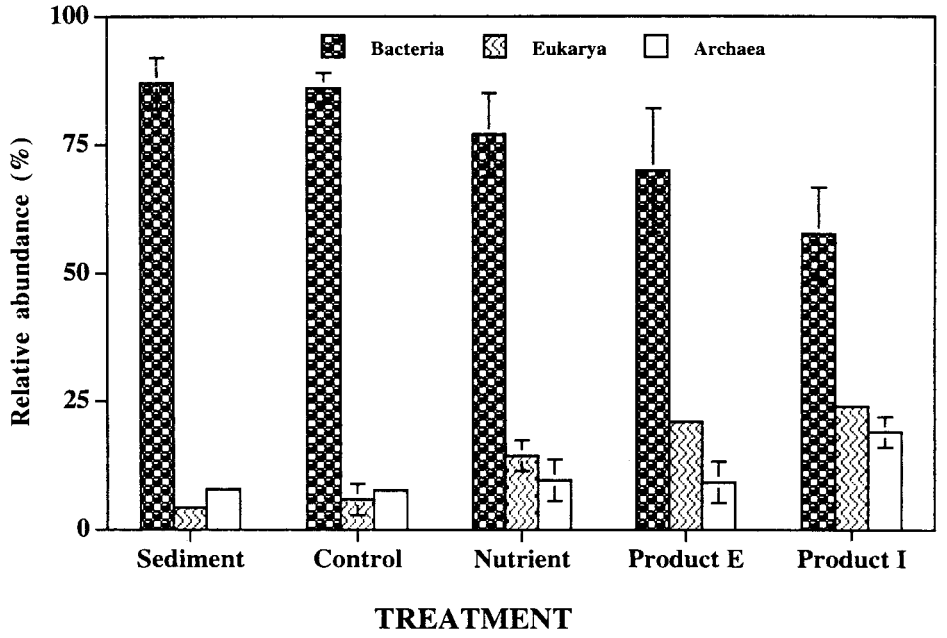

Figure 3 Relative abundance of the domains in the oil-impacted microcosm sediment treatments and initial sediment-extracted DNA samples, at 3 months. Hybridization signals were normalized to that of the universal probe.

and from those amended with Product I (5.9 μg/g) did not differ significantly ($P>0.05$) from the quantity extracted from the initial sediment (4.6 μg/g) (Table 3). In contrast, nutrient amendments (8.7 μg/g) and application of Product E (8.3 μg/g) both resulted in the extraction of greater amounts of DNA (Table 3).

Molecular analysis of the microbial community

Domain probe hybridizations indicated that the majority of the sediment DNA pool were bacterial DNA (Figure 3). Bacteria contributed (86–87%) to the DNA in the initial sediment and in the oil-impacted control microcosm (Figure 3). Lower proportions of bacterial DNA were found in other microcosm treatments, nutrient treatment (77%), Product E (70%), and Product I (57%), due to the concomitant increase in the amount of Archaea and Eukarya DNA in those treatments.

The relative percentage of Archaea DNA in the initial sediment (4%) and the oil-impacted control microcosm (5%) was similar to nutrient treatments (7%) and Product E (6%). In contrast, Archaea DNA in treatment I (21%) increased significantly (Figure 3). Eukaryotic DNA increased in all treatments relative to both the

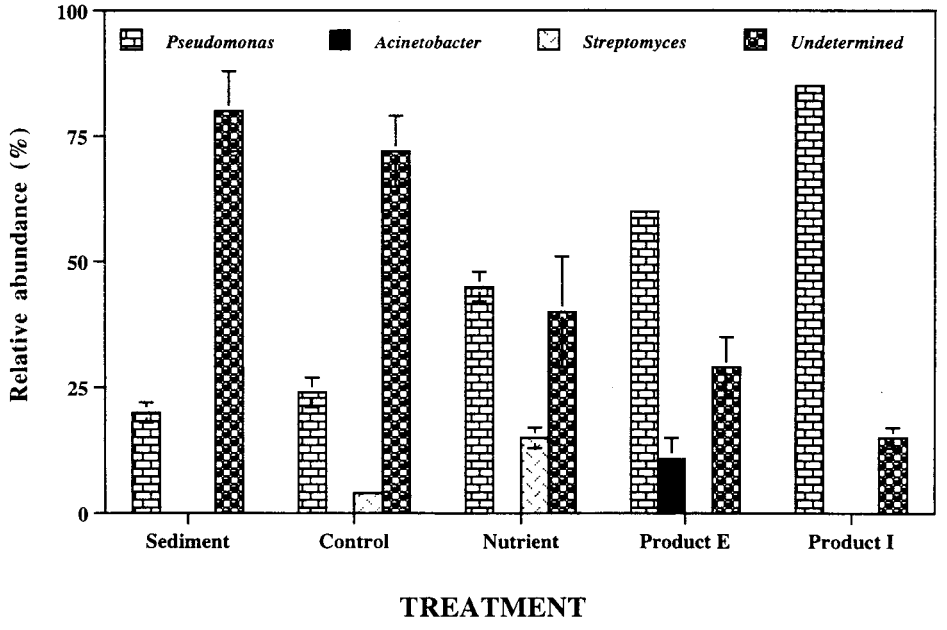

Figure 4 Relative abundance of the indicator bacterial groups in the microcosm sediment treatments and the initial sediment, at 3 months. Hybridization signals were normalized to that of the bacteria-specific probe.

initial sediment and the control treatment; the increase was highest with treatment I.

Group-specific probe analysis

DNA extracts from the microcosm sediments and Product E were hybridized with probes of three representative bacterial groups: *Pseudomonas* (alpha subgroup), *Streptomyces*, and *Acinetobacter*. Of the three groups of bacteria monitored, only *Pseudomonas* was detected in the initial sediment (20%) that was used to construct the microcosms (Figure 4). *Pseudomonas* was still detected in all treatments after 3 months, at approximately the same as initial levels in the control (24%) and at elevated levels in the other three treatments (N (45%), E (63%), and I (88%)). Neither *Streptomyces* nor *Acinetobacter* was detected in microcosms dosed with the surfactant (I); however, *Streptomyces* was detected at low levels in the controls (6%) and in treatment N (13.5%). *Acinetobacter* contributed 9% of the community DNA pool in treatment E (Figure 4) but was not detected in any of the other treatments. Furthermore, hybridization analysis of Product E DNA extract detected *Acinetobacter* (~5%) and *Pseudomonas* (~4.5%) DNA, but *Streptomyces* was not detected (data not shown). Therefore, *Acinetobacter* was used as an indicator bacterial group for the presence of bacteria introduced to the microcosm treated with Product E.

Discussion

Stimulation of the indigenous sediment microbial populations with nutrient amendments resulted in the greatest extent of PHC degradation (Figure 2). The enhancement of degradation was most pronounced for alkane hydrocarbon components, which were markedly reduced compared to controls. The aromatic constituents of PHC were reduced by half, at most, compared to controls. Product E was the second most effective bioremediation treatment. On a weekly basis, Product E added (18.2 mg N and 1.8 mg P) to the sediment and sediment dosed with nutrient received (13.6 mg N and 10.5 mg P), making it possible for the nutrient component of E to contribute to its effectiveness.

Atlas and Bartha [3] described maximal rates of biodegradation occurring at nutrient amendments of 0.13 mg N/g oil and 0.01 mg P/g oil. Prince *et al* [19] reported enhanced degradation of surface and subsurface oil on the shores of Prince William Sound in response to applications of polymerized fertilizer (Customblend). The enhanced degradation could not be unambiguously associated with increases in total bacterial numbers, but was related to increases in the fraction of oil degraders and with increases in mineralization rates for specific hydrocarbons [6].

The ratio of the straight- and branched-chain alkanes has been used as an indication of biological rather than chemical degradation of oil, although the usefulness of the ratio is limited to early stages of biodegradation because the branched forms are eventually degraded [19]. In the oil-impacted sediments, the divergence of the C17:pristane and C18:phytane ratios from those of the parent oil indicates that the oil had been biologically altered in all cases, with nutrients and Product E having reduced those ratios to a greater extent than Product I or the controls (Table 2).

Aromatic compounds were degraded to a lesser extent than were aliphatics (Figure 2B). Although the nutrient additions increased degradation of these compounds relative to the control, Product E gave mixed results and Product I inhibited degradation of aromatics, except for the dibenzothiophenes and pyrenes. The inhibitory effect of the surfactant product has been noted under field and laboratory conditions [7,15]. Surfactant may suppress degradation either by inhibiting the growth of bacteria or by functioning as an alternate, preferred substrate.

Bacterial plate counts were consistent with numbers reported for other coastal sediments as was the lack of difference between oiled and unoiled sediments [4]. However, it is likely that plate counts underestimated the total numbers of bacteria in the sediment. The validity of plate counts as an indicator of total bacteria has been widely questioned. Plate counting generally underestimates the numbers of bacteria by as much as 100-fold [12] and may obscure differences between treatments.

The MPN technique indicated that the addition of nutrients and Product E increased the numbers of oil degraders in the sediment relative to the initial and oil-impacted control. However, the higher numbers of oil degraders associated with Product E did not result in corresponding high rates of oil degradation. Product E degraded alkanes to an extent intermediate between the nutrients and the control and aromatics to a lesser extent than either treatment.

The amount of microbial DNA extracted from the sediments (Table 3) is a more accurate and responsive measure of the total numbers of bacteria present [4,23]. Microcosms amended with nutrients yielded the most DNA and also resulted in the greatest extent of oil degradation. Product E yielded an intermediate amount of DNA, and likewise resulted in an intermediate level of degradation. Results with Product I did not differ from those of the initial sediment or the control in the amount of DNA extracted.

Domain probe analysis (Figure 3) indicated that eubacteria dominated the sediment microbial communities. The values reported here are consistent with previous analyses of the Sapelo Island sediment community, which indicated that bacteria comprised 70–80% of the microbial community [4]. Archaea increased in all treatments except the control. The most pronounced increase in Archaea was in the microcosms treated with Product I (26%). The increase in Archaea was not reflected in enhanced oil degradation, as evidenced by a like increase in the fraction of eukaryotic DNA associated with Product I. Rather, the increases in degradative activity were consistent with increases in (eu)bacterial DNA, as reported frequently [2,3].

The use of group-specific probes enabled us to assess the fate of certain indigenous and introduced bacteria in oiled microcosms. *Acinetobacter* was detected only in microcosms dosed with Product E. Therefore, it is likely that the presence of *Acinetobacter* (9%) only in treatment E but not in any of the other treatments was due to bacteria introduced with that product. Although these bacteria survived and grew in the microcosm, their presence did not enhance oil degradation.

Pseudomonas, a group that has been widely associated with degradation of oil [2,3,28], increased in response to oil contamination of the sediment. However, the most effective treatment was N; it had a lower proportion of *Pseudomonas* (45%) than treatments E and I. The most dramatic increases in *pseudomonades* were associated with Product I, which did not increase oil degradation. Therefore, oil degradation cannot be attributed unambiguously to the group.

Although *Streptomyces* were not detected in the initial sediment (Figure 4), their presence, as 2–5% of the bacterial rRNA, has been reported in Sapelo Island sediments [14]. The *Streptomyces* populations detected in treatments C and N were likely derived from growth of previously undetected organisms. The relatively

high proportion of *Streptomyces* in treatment N, compared to the other microcosm treatments and the enhanced PHC degradation in N, suggests that these bacteria respond to oil contamination. However, given that more than 70% of the microbial DNA in the initial sediment and in the control microcosms, and nearly 40% of that in treatment N — the most effective treatment — was uncharacterized, it is likely that additional oil-degrading organisms were active but undetected.

Addition of inorganic nutrients was the most effective means of enhancing PHC degradation in the salt marsh sediments. Of the community measures tested, the enhancement of oil degradation was most strongly related to increases in extracted DNA. The degradative activity was associated with an increase in *Streptomyces* and other uncharacterized bacterial groups. Bioaugmentation resulted in the long-term survival of introduced bacteria, but those bacteria may not necessarily manifest oil-degrading activity under conditions of competition with indigenous microorganisms. Thus, microcosm assessment of bioremediation strategies of oil-contaminated salt marsh sediment by chemical and biological analyses provided insights into community population dynamics and the efficacy of applied bioremediation technologies.

Acknowledgements

This research was partly supported by a grant from the National Network for Environmental Management Studies (NNEMS) graduate student fellowship program and the Department of Energy (DE-FG02-97ER62451).

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