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Effect of inoculation on the biodegradation of weathered Prudhoe Bay crude oil

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SUMMARY

Enrichment cultures from oil-contaminated beach material from Prince William Sound, Alaska, generated both a mixed bacterial community of indigenous, oil-degrading marine microorganisms and a pure culture oil-degrader, strain EI2V. The mixed and axenic cultures were used in comparative shake flask studies of inoculation on biodegradation of Prudhoe Bay crude oil. Within 12 h following inoculation of homogenized, oiled beach material with the mixed culture, total CO_2 production was increased 2-fold relative to a noninoculated control. Moreover, measurements of phenanthrene degradation (as determined by the release of $^{14}CO_2$ from [9-^{14}C]phenanthrene) showed a 2- or 3-fold greater degradation when inoculated with either strain EI2V or with the mixed culture, respectively. However, as medium was replaced by a simulated tidal cycle, the observed stimulation of CO_2 production decreased, and the addition of strain EI2V had no greater effect on total CO_2 production than the addition of inorganic nutrients alone. Chemical analysis of oil recovered after 7 days incubation also suggested that, while these cultures are capable of efficient biodegradation of Prudhoe Bay crude in liquid culture, inoculation of beach material with high numbers of these microorganisms had little effect on the rate and extent of biodegradation of weathered crude oil. Overall, the sustained stimulatory effect was no greater than that observed with the addition of inorganic nutrients alone.

INTRODUCTION

The grounding of the Exxon Valdez on March 24, 1989, resulted in the release of approx. 42 million liters of Prudhoe Bay crude oil into the waters of Prince William Sound (PWS), Alaska. Assuming that 10% or less of the spilled oil was recovered [18], that 35% evaporated, and that 15–25% washed out of PWS, it has been estimated that 16.7 million liters of oil (40% of the total spill) ultimately contaminated over 188 km of coastline of PWS by the end of the summer of 1989 [10,14].

Once oil contaminates beach materials, its fate is determined by a variety of biotic and abiotic factors [10]. Whereas a majority of the hydrocarbons present in crude oil are biodegradable [1,3,15,22], biotic factors play a major role in the removal of oil contamination. More specifically, biodegradation of crude oil has been shown to occur under Arctic conditions [2,5], and several oil-degrading, psychrophilic, halophilic microorganisms have been isolated and characterized [5,7,21].

Because biodegradation represents a major route by which oil and its associated hydrocarbons are removed from contaminated environments, bioremediation emerged as a promising alternative clean-up technology for restoration of oil-contaminated beaches in PWS [20]. In oil-contaminated aquatic environments, oil-degrading microorganisms are likely to become substantially enriched leading to a situation where availability of nitrogen and phosphorus becomes the primary factor limiting the in situ rate of oil biodegradation [1,3,4,8,11]. It appeared logical, therefore, that addition of these elements to oil-contaminated beaches might possibly enhance in situ oil biodegradation. Such nutrient additions were indeed shown to stimulate the activity of indigenous oil degraders and increase the rate of oil biodegradation by 3- to 5-fold in PWS during the summer of 1990 [20].

Inoculation of oiled beaches with oil-degrading microorganisms has also been proposed as a generic bioremediation approach to ameliorate materials contaminated with organic chemicals. Previous studies have provided some support for this approach by demonstrating that, under certain conditions, inoculation accelerated the rate

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and extent of oil biodegradation [9,12,13]. However, in situ inoculation of PWS beaches was not considered initially because of data showing that high numbers of indigenous, oil-degraders were present on these beaches, and that these microorganisms were actively degrading oil (R.G. Prince, J.R. Clark and J.E. Lindstrom, Bioremediation Monitoring Program, December, 1990). Moreover, laboratory and field studies showed that the treatment of oiled beaches with fertilizers increased the number of oil-degrading microorganisms and their hydrocarbon mineralization potentials [16,17].

Nevertheless, to assess further the potential of in situ inoculation as an alternative bioremediation treatment for weathered oil present on beach material in PWS, laboratory experiments were initiated with the following objectives: (i) to determine the activity of indigenous oil-degrading microorganisms present on oiled beach material collected from PWS; and (ii) to ascertain whether inoculation with either mixed or axenic bacterial culture of oil-degraders could accelerate the rate, or further the extent, of oil biodegradation beyond that caused by the addition of inorganic nutrients alone.

MATERIALS AND METHODS

Biometer flask studies

Biometer flasks (250 ml; Bellco Biotechnology, Vineland, NJ) were used to measure CO_2 production as a measure of the activity of indigenous, oil-degrading microorganisms in a manner similar to that originally described by Bartha and Pramer [6]. Exactly 100 g of homogenized (sieved, <12.5 mm, >4.75 mm), uniformly oiled (0.4% w/w) beach material (coarse mixed sand and gravel) freshly collected from Disk Island, PWS, Alaska (located on the east side of Eleanor Island), was added to each biometer flask.

To simulate tidal conditions similar to those of PWS, oiled beach materials were treated in an artificial tidal cycle. To facilitate tidal cycling while maintaining the integrity of the semi-closed system, the biometer flask was modified by placing a beveled, Teflon tube fitted with a swivel lock and plugged with a 10-ml hypodermic syringe through the rubber stopper holding the Ascarite trap and extending it to the bottom of the biometer flask (Fig. 1). A 12-h 'high-tide' period was simulated by adding, through the Teflon tube, 50 ml of a sea water solution (see below) which was of sufficient volume to submerge all beach materials in the flask.

Flasks were incubated at 15 °C in the dark and wave action was simulated by gentle mixing (75 rpm). For 'low-tide' conditions, a 60-ml hypodermic syringe was connected to the Teflon tube, the bulk ($>95\%$) of the aqueous solution withdrawn, and flasks were further incubated for 12 h. Each volume of tide water was saved to determine the amount of oil residue physically removed from the test systems. At each tidal change, NaOH trapping solutions in the side-arm were sampled to measure total CO_2 released (see Microbial activity measurements). This procedure was repeated as necessary to stimulate the requisite number of tidal cycles. While the use of fresh beach material provided indigenous microbial populations adapted to the oil contamination and the tests systems were designed to model the natural environmental conditions, it is realized that the duration of the experiments

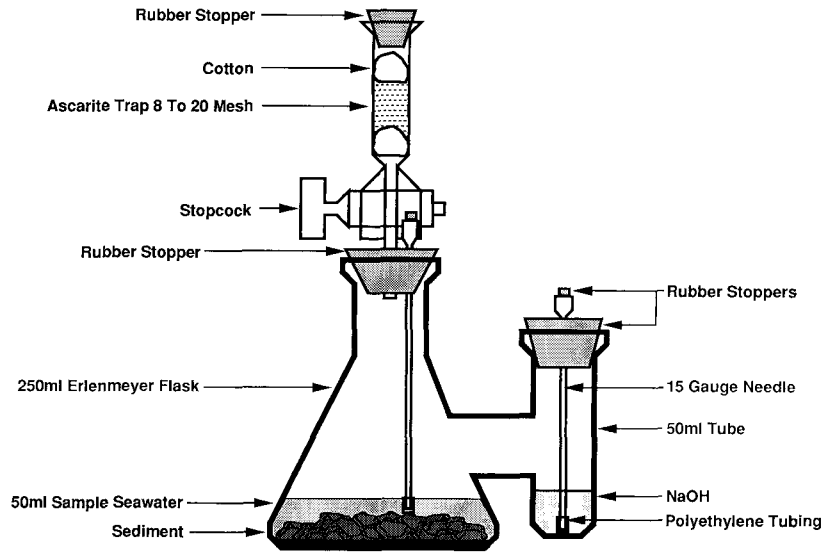

Fig. 1. Modified Biometer flask used for measurement of microbial activities.

provided an evaluation of these populations after a period of ex situ selection and growth that may be considered limited.

Nutritional amendments

Indigenous, oil-degrading microorganisms associated with oiled beach material were exposed to inorganic nutrients by amending filtered sea water that was used for high-tide simulation. Fresh PWS sea water was filtered (0.7-μm glass fiber filter) and supplemented with 3.6 mmol N as NH_4Cl (35 ppm N) plus 0.8 mmol P as KH_2PO_4 (7 ppm P). Treatments were applied daily with each high-tide to duplicate biometer flasks. Resultant microbial activities were compared with those observed when the high-tide solutions consisted of unamended, filtered PWS water.

Isolation of bacterial cultures

Beach material contaminated with weathered Prudhoe Bay crude oil was collected from Disc Island and Elrington Island in Prince William Sound, Alaska. These materials were used as a source of inoculum to establish an enrichment culture at 15 °C in Bushnell-Haas (BH) medium containing 2% NaCl, 0.5% Prudhoe Bay crude oil (autoclaved) and 0.02% dimethylsulfoxide (DMSO). The resulting mixed culture grew to a high cell density within 4 days of incubation with shaking (200 rpm) and was routinely transferred (2% inoculum) to identical medium at weekly intervals.

When subcultures of this community were plated onto complex medium (Luria agar containing 2% NaCl), five morphologically distinct bacteria were isolated: strains DI/EI 1, 2, 3, 4 and 5. In addition, when subcultures were streaked onto carbon-free BH + 2% NaCl agar and sprayed with an ethereal solution of phenanthrene (0.2%), zones of clearing of the hydrocarbon substrate indicated their ability to utilize phenanthrene as a primary carbon source. Following 1–2 weeks incubation at 15 °C, this procedure resulted in the isolation of a phenanthrene-degrading bacterium identified as strain DI/EI 4.

Additionally, a sixth isolate, strain EI2V, was obtained directly from oiled beach material (collected from Elrington Island) by plating on BH + 2% NaCl agar containing 0.2% Prudhoe Bay crude oil. Subsequent studies showed that this strain grew to high density in BH + 2% NaCl broth containing 0.5% Prudhoe Bay crude oil. This isolate also rapidly dispersed or emulsified the oil when grown in liquid medium (unpublished data).

Microbial amendments

Two inocula, one of strains DI/EI 1, 2, 3, 4 and 5 and one of strain EI2V were prepared from subcultures by inoculating two, 250 ml Erlenmeyer flasks containing 75 ml of sterile BH medium plus 2% NaCl, 0.02% DMSO and 0.5% autoclaved Prudhoe Bay crude oil. Cultures were grown for 4 days at 15 °C with shaking (200 rpm), harvested by centrifugation (4 °C, 8000 rpm for 10 min) and the resulting cell pellets were resuspended in 100 ml of filtered PWS water amended with 35.7 ppm N and 8.07 ppm P. Cell densities of the defined mixed culture and strain EI2V corresponded to an A_{600} of 2.07 and 1.17, respectively. Aliquots (50 ml) of these suspensions were added to duplicate biometer flasks to stimulate the first high tide. The inoculation resulted in the addition of approx. 1×10^7 and 1×10^6 oil-degrading microorganisms/g beach material for the mixed culture and strain EI2V, respectively. No carry over of oil hydrocarbons resulted from this procedure (data not shown).

Microbial activity measurements

Evolved CO_2 was trapped in 10.0 ml of a 0.5-M NaOH solution (prepared with CO_2-free water) located in the side-arm of the biometer flask. At each 12-h interval, NaOH was removed from the biometer flask, placed in a 25-ml Wheaton bottle and crimp-sealed with a butyl rubber stopper. Fresh trapping solution (10 ml) was placed in the side-arm at each sampling point. The amount of trapped CO_2 was determined by acidifying (pH < 2.5 with 8.5% phosphoric acid) NaOH samples and analyzing headspace gases by gas chromatography as described below (see Analytical methods). Background concentrations of CO_2 were determined for each sampling point.

After monitoring microbial CO_2 production rates for 72 h, radiolabeled [9-^{14}C]phenanthrene (spec. act. = 13.1 mCi/mmol; Sigma Chemicals, St. Louis, MO) was used to define further the catabolic activities of oil-degrading microorganisms. Radiolabeled substrate (2.5×10^6 dpm/50 ml) was introduced with the fourth high-tide. Release of $^{14}CO_2$ was determined by liquid scintillation analysis of duplicate, 1.0-ml samples of the NaOH solutions recovered at 12 h intervals for a further 96 h of incubation.

The percentage of radiolabeled phenanthrene mineralized was calculated based on the amount of radioactivity adsorbed to the oil layer in each biometer flask. To determine the amount of radiolabel removed with the 'outgoing' tide, duplicate 1.0-ml samples of sea water were counted at the time high-tide was removed (12-h residence time). Preliminary studies with active samples and sterile controls demonstrated that within 6-h incubation at 15 °C with shaking (75 rpm), 10 to 15% of the radiolabel was washed from the system with a tidal flush and, therefore, 85 to 90% of the radiolabeled substrate was presumed to have become physically adsorbed to the oil layer coating the beach material. Similar values were consistently observed throughout these studies.

Oil residue analyses

At the conclusion of radiolabeled CO_2 study (7-day total incubation period), sea water was removed and 100 ml of methylene chloride was added to each biometer flask. Flasks were shaken (200 rpm) for 1 min, and the organic phase was transferred to a clean flask. This extraction procedure was repeated two more times each with 50-ml volumes of methylene chloride. Combined organic phases were passed through a layer of granular, anhydrous sodium sulfate (approx. 25 g) to remove residual water and suspended solids. Methylene chloride was removed under a stream of dry nitrogen at 25–30 °C, transferring the organic extracts to clean (methylene chloride-rinsed), tared 25-ml test tubes. Residual solvent was removed by placing tubes in vacuo in a desiccator for 48 h. Preliminary tests showed no significant weight loss through use of this technique. Tidal waters were extracted in a similar manner.

Material recovered by methylene chloride extraction was subsequently extracted three times with 10-ml volumes of hexane [19]. Separation of hexane-insoluble asphaltenes was facilitated by centrifugation (5000 rpm, 10 min). Hexane-soluble fractions were transferred to clean, tared test tubes and hexane volume was reduced under a stream of dry nitrogen at 30 °C. Residual solvent was removed from both the hexane-soluble and hexane-insoluble fractions in vacuo. Extracts from tidal waters were combined with those from the beach materials, and changes in the hydrocarbon composition of these extracts were determined by gas chromatographic analysis (see Analytical methods).

Analytical methods

Carbon dioxide was determined with a Hewlett-Packard model 5890 gas chromatograph headspace gas analyzer fitted with a Ni catalyst methanizer and a flame ionization detector. Duplicate, 100-μl samples of headspace gas from acidified (pH < 2.0) NaOH trapping solutions were injected onto a fused silica Chrompack Poraplot Q stationary phase column (27.5-m length; 0.53-mm i.d.; Supelco, Bellafonte, PA). Injector and detector temperatures were maintained at 220 and 250 °C, respectively. Hydrogen (150 kPa) was used as the carrier gas. Quantitation of CO_2 was based on a 6-point standard curve. Sampling precision (relative percent difference between sample replicates) averaged 7% and ranged between 0.1–20%.

Aliphatic hydrocarbon composition of the oil of the hexane extracts was determined with a Hewlett-Packard model 5890 series II gas chromatograph equipped with dual injection ports, dual columns, an autosampler and dual flame ionization detectors (FID). Helium (150 kPa) was used as the carrier gas (linear velocity 48 cm/s), while air (250 kPa) and hydrogen (150 kPa) were supplied for the flame ionization detector. Oil constituents were separated using a SPB-5 capillary column (15 m × 0.32 mm i.d. with a 0.25-μm film thickness; Supelco, Bellafonte, PA). The temperature program was as follows: 35 °C for 3 min followed by a linear increase of 5 °C/min to 300 °C where it was held for 6 min. Injector and detector temperatures were maintained at 300 °C and 310 °C, respectively. Hydrocarbon concentrations were calculated by comparing peak area obtained by duplicate, 1.0-μl injections against peak area of standards of each hydrocarbon and related to the amount of an internal standard (naphthalene). Linear aliphatic (*n*-C18, *n*-C21) and branched hydrocarbons were monitored specifically. Given the chemical complexity of the hexane extracts, however, data are presented as total area of all peaks having a height of 10% or more of the detector response (limit of detection = 100 ppb). Sampling precision averaged 5% and ranged between 0.3–11%.

Measurements of radioactivity were obtained by adding 10 ml of liquid scintillation cocktail (Ready-Safe, Beckman Instruments) to duplicate, 1.0-ml samples of alkaline trapping solution in glass scintillation vials. Vials were allowed to equilibrate in the dark for 24 h prior to counting. Counts were obtained with a Beckman model 8100 liquid scintillation counter (sigma factor = 2). Sampling precision averaged 1% and ranged between 0.1–2%

RESULTS

Physiological and catabolic traits of enriched microorganisms are summarized in Table 1. All of the bacteria isolated were tentatively characterized as species of *Rhodococcus* or *Pseudomonas* as determined by lipid profile analyses (Microbial ID, Inc., Newark, DE). Given the relatively small amount of microbiological diversity, at least at the genus level, it is presumed that the enrichment processes employed strongly influenced the outcome of the selection efforts. Although used successfully, the presence of 0.02% DMSO as a solubilizing agent to help select for microorganisms capable of degrading the more persistent (less water soluble) components of crude oil may have contributed to the selective action observed. Nevertheless, a number of microorganisms (strains DI/EI 1, 2, 3, 5 and EI2V) capable of rapid growth with crude oil and individual *n*-alkanes in the absence of DMSO were obtained. Since such growth processes are known to involve the production of biological surface active agents, the use of DMSO did not seem to counterselect against such microorganisms.

Under shake flask conditions, both the mixed culture and strain EI2V extensively degraded autoclaved (artificially weathered) Prudhoe Bay crude oil constituents, in-

TABLE 1

Organisms isolated from oiled beach material in Prince William Sound, Alaska

Organism designation	Enriching substrate	Tentative identification[b]	Carbon source tested as growth substrates[a]					
			oil	*n*-C8	*n*-alkanes[c]	pristane	naph	phen
DI/EI 1	crude oil	*Rhodococcus* sp.	+	+	+	+	–	–
DI/EI 2	crude oil	*Rhodococcus* sp.	+	+	+	+	–	–
DI/EI 3	crude oil	*Pseudomonas* sp.	–	+	–	–	–	–
DI/EI 4	crude oil/phenanthrene	*Pseudomonas* sp.	–	–	–	–	–	+
DI/EI 5	crude oil	*Rhodococcus* sp.	+	+	+	+	–	–
EI2V	crude oil	*Rhodococcus* sp.	+	+	+	+	–	–

[a] *n*-Octane (C8), *n*-dodecane (C12) and naphthalene (naph) were supplied to plates (BH + 2% NaCl) in vapor phase; *n*-hexadecane (C16), *n*-eicosane (C20), *n*-hexacosane (C26) and phenanthrene (phen) were added to Bushnell-Haas + 2% NaCl broth at 0.1%; Prudhoe Bay crude oil was added to broth at 0.2%. Plates and broths were incubated at 15 °C (with shaking at 200 rpm where appropriate) for 1 week and scored against inoculated, carbon-free controls.

[b] Tentative genus identification based on fatty acid profile analysis (Microbial ID., Inc., Newark, DE).

[c] *n*-Alkanes include dodecane, hexadecane, eicosane and hexacosane.

cluding pristane and phytane (Fig. 2). Therefore, inoculation studies were initiated to compare the abilities of both a mixed and axenic bacterial culture to stimulate oil biodegradation above and beyond that resulting from the addition of inorganic nutrients alone.

The production of CO_2 (Fig. 3) was used to measure the effect of treating oil-contaminated beach material with these organisms, applied in combination with inorganic nutrients, and compares these data with those obtained with nutrient-enhanced oil biodegradation. The highest microbial activity was observed after 12-h incubation in flasks inoculated with the mixed culture: the amount of CO_2 released during this period was twice that observed in the other treatments. Beyond the initial 12 h of incubation, similar rates of CO_2 production appeared for all flasks

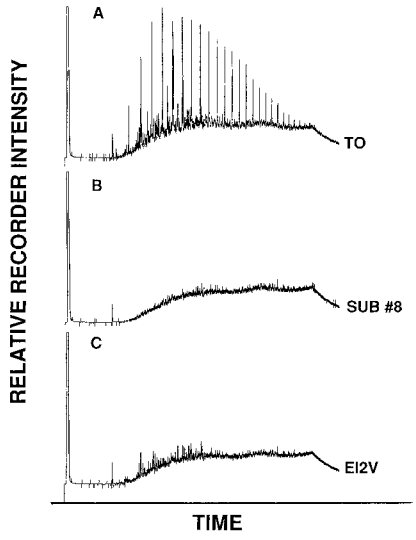

Fig. 2. Capillary gas chromatographic analyses of hexane extracts of Bushnell-Haas medium containing 2% NaCl and 0.2% Prudhoe Bay crude oil after 10 days incubation (15 °C, 200 rpm) showing action of a defined mixed culture (B) and strain EI2V (C) towards autoclaved Prudhoe Bay crude oil under shake flask conditions compared to a noninoculated control (A).

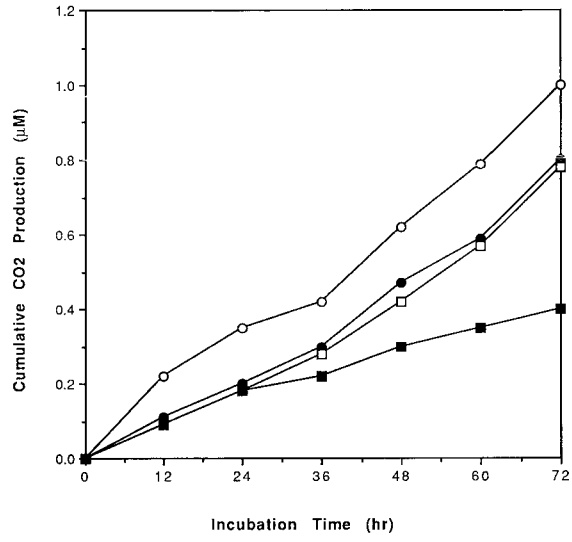

Fig. 3. Effect of inorganic nutrients and microbial supplements on the release of CO_2 from oiled beach material in biometer shake flasks. Production of CO_2 following treatment with: mixed culture + soluble inorganic nutrients (○), strain EI2V + soluble inorganic nutrients (●), soluble inorganic nutrients alone (□) and unamended PWS water (■).

except for the controls hence this stimulatory effect diminished with time (see Discussion). Statistical analysis performed by linear regression analysis on the slopes of regression functions (mixed culture plus nutrients slope = 0.013, $r^2 = 0.99$; EI2V plus nutrients slope = 0.011, $r^2 = 0.98$; nutrients alone slope = 0.011, $r^2 = 0.97$) showed that the amount of CO_2 liberated with these treatments was significantly greater ($P = 0.10$, GLM) than that produced in the unamended controls (slope = 0.05, $r^2 = 0.99$). However, statistically significant differences between treatments were not observed ($P < 0.10$).

Assuming that most of the added microorganisms were removed with the outgoing tide, the data suggest that the observed response may be due to CO_2 production from the large number of cells initially added to the system; that is, the CO_2 generated in the systems was not necessarily a consequence of oil mineralization. Considering the slopes of the lines generated from plotted data, it is apparent that the effect of adding high numbers of oil-degrading microorganisms, in association with inorganic nutrients, was not substantially greater than that resulting from the addition of soluble nutrients alone. In the absence of both chemical and biological amendments, however, microbial respiration was much lower.

Analyses of the chemical composition of recovered oil support the conclusion that microbial inoculation had little effect on the biodegradation of autoclaved Prudhoe Bay crude beyond that resulting from the addition of inorganic nutrients alone (Table 2). When compared to the noninoculated, unamended control receiving filtered PWS water, 69, 67 and 70% of the oil originally present was recovered after 7 days incubation in the systems receiving the mixed culture, strain EI2V or inorganic nutrients alone, respectively. However, the relatively short incubation period made it difficult to discern definitive differences.

TABLE 2

Changes in amounts of oil recovered (total peak area) from treatments as determined by gas chromatographic analyses of extracted beach materials

Treatment	Mean[a] total peak area (log_{10})	% remaining[b]
Mixed culture with soluble nutrients	6.20	68.8
EI2V with soluble nutrients	6.19	67.0
Soluble nutrients only	6.21	69.9
Noninoculated, unamended PWS water	6.24	75.8
Time-zero	6.36	100

[a] Mean of duplicate determinations of areas of peaks having 10% or more of detector response.

[b] % remaining is determined as a percentage of the amount of oil at time zero (400 mg oil/100 g beach material).

While CO_2 production and oil chemistry data suggest that inoculation had only a small effect on the measured parameters, the addition of either the mixed culture or strain EI2V, in association with inorganic nutrients, had a discernible affect on the rate of mineralization of radiolabeled phenanthrene when measured 3 days post-inoculation (Fig. 4). Within 12 h of substrate addition, 23% and 12% of the labeled phenanthrene was mineralized in flasks inoculated either with the mixed culture or with strain EI2V, respectively. However, only 8% of the substrate was mineralized with nutrient-amended, noninoculated flasks, and only 2% phenanthrene mineralization was observed with the unamended treatments. These differences were most evident within the first 24 h of incubation. Linear regression analysis showed that the slopes of the regression functions associated with inocula and fertilization treatments were statistically different ($P = 0.05$, GLM) from the unamended control, but no statistically significant differences ($P < 0.10$) between treatments were observed.

Addition of strain EI2V enhanced the rate of [^{14}C]-phenanthrene mineralization despite the fact that this microorganism was unable to degrade phenanthrene (Table 1). One likely explanation for this response relates to the observed ability of strain EI2V to rapidly emulsify oil; namely, the addition of strain EI2V to the test systems

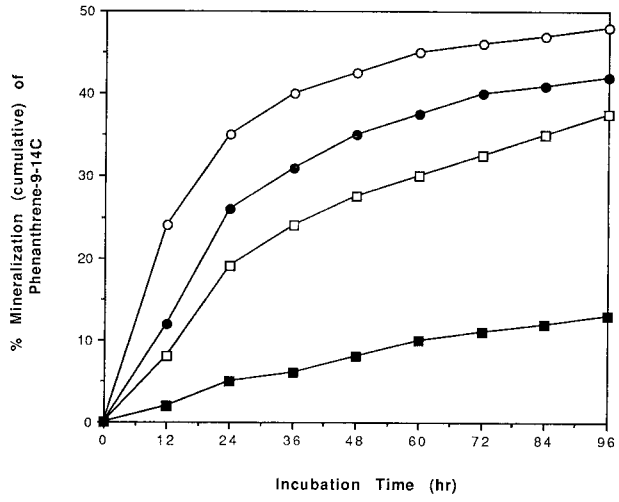

Fig. 4. Effect of inorganic nutrients and microbial supplements on the release of $^{14}CO_2$ from [^{14}C]phenanthrene added to oiled beach material in biometer shake flasks. Release of $^{14}CO_2$ following treatment with mixed culture + soluble inorganic nutrients (○), strain EI2V + soluble inorganic nutrients (●), soluble inorganic nutrients alone (□) and unamended PWS water (■).

resulted in the solubilization of oil residues which, in turn, enhanced the activity of indigenous, phenanthrene-degrading microorganisms associated with the oiled beach material recovered from PWS, Alaska. Likewise, the effect of inoculation with the mixed culture on the phenanthrene mineralization rates may be related to the action of surfactant-producing microorganisms growing at the expense of *n*-alkanes moreso than the addition of phenanthrene-utilizing strain DI/EI4 per se. Thus, advantages realized through inoculation efforts may primarily be the result of increased bioavailability through the biological production of surface active agents.

DISCUSSION

Upon initial inoculation of beach material with enriched, oil-degrading microorganisms under semi-continuous conditions, an increase of microbial activity, as determined by CO_2 production, was observed. This activity decreased to rates equivalent to those of the noninoculated, nutrient-amended controls. Washing out of cells associated with the simulated tidal cycling represents one explanation for this observation. Another possibility is that the mixed culture microorganisms oxidized endogenous energy reserves when the cells were introduced into the hydrocarbon environment. An alternative view of these results would suggest that the addition of inorganic nutrients rapidly enhanced the activity of the indigenous microbial population to levels equivalent to those observed after inoculation with high levels of oil-degrading microorganisms.

The lack of a significant, sustained, stimulatory response may have been due to inability of the added microorganisms to attach to the beach material. Possibly, the use of different inoculation techniques (e.g., application at low tide) may have yielded a better response. However, in the absence of a commercial preparation specifically designed for field application, bathing oiled beach material with aqueous suspensions of medium containing high cell numbers provided for close contact between inoculant microorganisms and their targeted substrate for at least a 12-h period of time. Alternatively, the use of microorganisms with different adherence capabilities may also have improved the observed response. For example, microorganisms obtained by enrichment methodologies designed to enhance growth of surface-attached biomass might prove to be more successful in adhering to beach surfaces.

To simplify chemical analyses, oil constituents recovered from tide waters were combined with those obtained from beach materials. While this approach allowed for an accurate determination of biodegradation, it was not possible to identify treatments that enhanced physical removal (e.g. emulsification, solubilization). Likewise, differences were not discerned in the chemical profiles of oil extracted from beach materials and that recovered from effluent waters. It can be stated, however, that the amount of 'oil' washed out of the systems was usually less than 1%, based on data obtained using radiolabeled phenanthrene as a tracer compound.

Overall, microbial CO_2 production and oil recovery data suggest that attempts to inoculate oil-contaminated beaches of PWS with the cultures tested may be of limited usefulness. The potential effect of such measures seems to be related to the increased bioavailability phenomena, and would presumably be limited by the ability of inoculant to remain in association with the targeted substrates. In this capacity, different inocula formulations may prove more effective. Given that the inoculated test systems exhibited elevated activity towards phenanthrene at least 3 days post-inoculation, this suggests that inoculation may have some potential to increase the bioavailability of certain inorganic compounds and thereby accelerate the rate and extent of oil biodegradation. Nevertheless, the sustained stimulatory effect of inoculation was no greater than that observed with the addition of inorganic nutrients alone.

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