Biodegradation of Dispersed Oil Using COREXIT 9500

A Report Produced for
The Alaska Department of Environmental Conservation
Division of Spill Prevention and Response

by
Jon E. Lindstrom[1]
Daniel M. White[2]
Joan F. Braddock[1]

[1]Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775
[2]Institute of Northern Engineering, University of Alaska Fairbanks, Fairbanks, AK 99775

30 June 1999

Executive Summary

Our study examined the effects of Corexit 9500 and sediment on microbial mineralization of specific aliphatic and aromatic hydrocarbons found in crude oil. Additionally, we measured gross mineralization of crude oil, dispersed crude oil and dispersant by our marine consortium in the absence of sediment. The only available previous study regarding the effects of Corexit 9500 on biodegradation of oil under simulated marine conditions (Swannell *et al.*, 1997) suggested that addition of dispersant stimulated biodegradation of oil in the water column. Our findings are consistent with the previous study's results, though our methodology has yielded data suggesting a somewhat different conclusion. The previous study examined the effects of nutrients on dispersion and microbial degradation of dispersed oil, while our study focussed on the effects of dispersant on oil biodegradation and did not specifically address nutrient limitation. The resolution of some of our assays provided data suggesting that dispersant-enhanced oil mineralization may be restricted to particular components of the crude oil. Specifically, we found that relatively less soluble hydrocarbon substrates were not metabolized by our test microbial consortium when dispersant was present, compared to their biodegradation when dispersant was not added to the assay (i.e., crude oil only used). Thus, while adding dispersant may increase microbial oil degradation activity as a whole, this increase may be restricted to only some components of the crude oil, resulting in selective enrichment of other components in the residual oil. This could result in either an increase or a decrease in the toxicity of the residual oil.

We recommend that this study be extended to include components of the previously reported work on Corexit 9500, incorporating the higher resolution assays used in this study. Specific recommendations for the future study's experimental design are provided in the Conclusions section of this report.

Contents

Introduction

Chemical dispersants have the potential to be effective tools in the management of crude oil spilled in the marine environment. However, several important factors must be considered in deciding whether to use chemical dispersants. One factor is the physical effectiveness of the dispersant under the conditions of expected use (e.g., temperature). Another is the toxicity of the dispersant and dispersed oil to local marine species. Researchers at the University of Alaska Fairbanks, Institute of Northern Engineering have performed studies addressing these first two factors. Some of this work has already been presented publicly (i.e., Rhoton *et al.*, 1998; Rhoton *et al.*, 1999). A third important issue is the effect of chemical dispersants on the fate of spilled petroleum hydrocarbon products. Biodegradation is the major mechanism for removal of spilled petroleum hydrocarbons from the marine environment. For example, Wolfe *et al.*, (1994) estimated that from the time of the *Exxon Valdez* oil spill in 1989 until the fall of 1992, about 50% of the spilled oil was biodegraded either in the water column or in the intertidal sediments. Thus, understanding the effect of any treatment strategy on the microbial degradation of oil is essential for predicting the long-term persistence of petroleum hydrocarbons in the environment.

Several research groups have examined the effects of adding dispersants, or more commonly, defined surfactants, on the biodegradability of various fractions of oil. Most of these studies have examined non-ionic or ionic surfactants, or bio-surfactants (those produced by microorganisms). A few researchers have examined commercial dispersant products such as Corexit 9527 (Foght and Westlake, 1982); Corexit 7664 (Rittman and Johnson, 1989); and Finasol OSR-5, Inipol IPF and Corexit 9527 (Bruheim *et al.*, 1997). The results of the various commercial dispersant studies were specific to the product used, with some dispersants increasing and some diminishing biodegradation. In addition, the mixed results appeared to be dependent on the specific hydrocarbon fraction examined. Foght and Westlake (1982), for example, found that Corexit 9527 affected alkane degraders differently than aromatic degraders. In addition, they found that the abundance of nutrients (i.e., nitrogen and phosphorus) influenced dispersant effects on petroleum biodegradation. Dispersant use may lead to diminished biodegradation if the dispersant contains components toxic to the microbial population. However, dispersants also may enhance biodegradation of bulk crude oil by increasing the bioavailable fraction of hydrocarbon. This is accomplished through mobilization of adsorbed hydrocarbon or by increasing its effective aqueous solubility (Tsomides *et al.*, 1995). Two

published studies examined biosurfactants during the clean-up of the *Exxon Valdez* oil spill. The first study was restricted to testing the physical effectiveness of a biosurfactant in a simple laboratory study (Harvey *et al.*, 1990). The second study, conducted in the field, examined both the physical effectiveness of the biosurfactant tested (PES-51) and the resulting effect on the population of hydrocarbon-degrading microorganisms (Tumeo *et al.*, 1994). In that field study the numbers of hydrocarbon-degrading microorganisms were stimulated temporarily after the clean-up, presumably due to mobilization to the surface of previously buried oil. Work with the dispersant Corexit 9500 recently has been reported (Swannell *et al.*, 1997). The authors found that Corexit 9500 increased the surface area of crude oil (increased numbers and smaller sizes of oil droplets in aqueous suspension), increased numbers of hydrocarbon-metabolizing microbes, and increased overall carbon mineralization. To our knowledge there are no published studies on Corexit 9500's effect on biodegradation of specific petroleum components in crude oil.

We used laboratory microcosm studies to examine how the addition of the dispersant Corexit 9500 affected the biodegradation of petroleum hydrocarbons under temperature conditions (8 °C) similar to those expected in a marine crude oil spill in southern Alaska. Since adsorption to marine sediments can limit biodegradation by decreasing the bioavailability of some hydrocarbons for microbial degradation (Braddock and Richter, 1997), we conducted some of the studies in sediment/seawater slurries. Using microbial communities harvested from petroleum-contaminated Alaskan marine sediments, laboratory microcosms were prepared to assess the biodegradability of aliphatic and aromatic hydrocarbons. The aliphatic hydrocarbons used were dodecane and hexadecane, common low and moderate molecular weight components of crude oil. Aromatic hydrocarbons included the polycyclic aromatic hydrocarbons (PAH) 2-methyl-naphthalene, phenanthrene, and pyrene. PAH are of particular interest as this group of compounds contains members that are known mutagens and carcinogens. In addition many PAH readily adsorb to marine sediments leading to long-term persistence of these compounds in the environment, a particular concern for oil dispersed into the water column. The results of our studies offer information on the effects Corexit 9500 may have on the biological degradation of components of crude oil (as opposed to bulk oil mineralization) in an Alaskan oil spill scenario.

Methods and Materials

Overview

We performed several experiments designed to assess the effects of Corexit 9500 on the biodegradation potential of crude oil in laboratory microcosms. These efforts can be broadly divided into three categories: 1) substrate-specific hydrocarbon mineralization assays using ^{14}C radioactively-labeled substrates (radiorespirometry); 2) total carbon mineralization assays using dispersant, oil, and combinations of the two; and 3) enumeration assays. All assays were incubated aerobically at 8°C. Additionally, a two-month-long oil degradation experiment was performed with fresh oil or dispersed oil in nutrient-amended media, using inoculated and sterile control flasks.

We conducted our microbiological assays using a consortium of marine microbes selected for their ability to degrade components of crude oil. Since several laboratory experiments were to be performed, we wished to assure that measured treatment effects were due to the variables (dispersant and sediments) being tested, and not due to variation in microbiota across experiments. Therefore we grew and froze a microbial consortium and used this as an inoculum in all experiments for this study. Early experiments included sediments as a variable in their design but, as this variable showed no significant effect for our defined system, sediments were omitted from later experiments. Details regarding selection and use of our consortium, and experimental design are provided below.

Microbial Consortium Inocula

All microbial inocula used in the various assays were prepared from a frozen source stored at -80°C. This assured that each experiment was controlled as much as possible with respect to microbial community composition and size. The frozen source was prepared from batch enrichment cultures using an inoculum originally derived from the *M/V Kuroshima* bunker C fuel spill that occurred near Dutch Harbor, Alaska in November 1997. This environmental inoculum was chosen because we had previously noted its ability to metabolize both alkane and aromatic hydrocarbons in laboratory assays. The frozen source material was prepared by inoculating 500 ml sterile marine mineral nutrient broth (Bushnell-Haas broth, or BH; Atlas,

1993) with one ml of an enrichment culture of *Kuroshima*-contaminated beach sand grown on BH and chitin. The transfer culture of BH broth was amended with *ca.* 5 mg phenanthrene and *ca.* 180 mg Alaska North Slope (ANS) crude oil per L to provide selective pressure for hydrocarbon degrading microbes. This 500-ml broth culture was grown on a shaker table at room temperature (*ca.* 20°C) for one week, at which time 2 ml broth was aseptically transferred to a fresh liquid medium of the same composition. The new transfer culture was grown on a shaker table at room temperature for 72 hours, then frozen for use in all future experiments. The culture was frozen at -80°C in cryogenic storage vials after combining 0.5 ml with an equal volume of a sterile 1:1 mixture of glycerin and deionized water.

Cultures for most radiorespirometry assays were prepared by thawing one of the cryostorage vials, adding its contents to 1 L marine BH broth containing 5 mg phenanthrene and 180 mg ANS crude oil, and incubating the culture on a shaker for 72 hours. This 72-hour culture was then used to assess the effects of dispersant and sediment on oil biodegradation.

Experimental

Radiorespirometry

Microcosm studies, using 72-hour cultures prepared as described above, were performed to evaluate the effects of sediment and/or dispersant on biodegradability of specific petroleum fractions. Radiolabeled (^{14}C) hydrocarbon substrates were provided to the cultures and evolution of $^{14}CO_2$ was measured by established techniques (Brown *et al.*, 1991). Previously sterilized, 40-ml septum vials (I-Chem Research, Hayward, CA) containing 10 ml culture, treatment materials (e.g., sediment, crude oil, dispersant, dispersed oil) and added growth substrates were used as microcosms. For experiments including sediment as a treatment, one gram dry, sterile marine sediment (sandy material, low clay and organic matter content) from Resurrection Bay, Alaska was added to the vial. If oil or dispersed oil and sediment were together added as treatments, the oil material was first added to the sediment and culture was then added to the vial. After adding culture broth to the microcosm vessels, the vials were shaken vigorously by hand for 30 seconds to distribute dispersant, oil or dispersant plus oil throughout the medium. Depending on the experiment, dispersant was added to oil at the rate of 1:10 (w/w) or 1:20 (w/w). In “low oiling” experiments, *ca.* 14 mg Alaska North Slope crude oil (ANS) was added to each vial (nominal concentration of 1400 mg/L), and in “high oiling” experiments *ca.* 45 mg oil was added (nominal

concentration of 4500 mg/L). After the microcosms were constructed, 50 µl of a 2-g/L solution (in acetone) of radiolabeled hydrocarbon was added by syringe to each vial through the septum. The resulting initial concentration of added hydrocarbon was then 100 µg per vial (10 µg/ml culture broth; radioactivity *ca.* 50,000 dpm). Substrates used (Sigma Chemical Co., St. Louis, MO) included the alkanes dodecane (1-^{14}C-labeled) and hexadecane (1-^{14}C-labeled), and the polynuclear aromatic hydrocarbons (PAHs) 2-methyl-naphthalene (8-^{14}C-labeled), phenanthrene (9-^{14}C-labeled) and pyrene (4,5,9,10-^{14}C-labeled). Each treatment was replicated 5-fold, and killed controls were used to check for abiotic $^{14}CO_2$ evolution. Vials were incubated at 8°C for 96 hours (168 hours for pyrene), killed by adding NaOH to stop respiration, and assayed for $^{14}CO_2$ from hydrocarbon mineralization (Brown *et al.*, 1991). The concentration of $^{14}CO_2$ from hydrocarbons mineralized in the microcosms was converted to a mass basis and used to derive the mineralization rate potentials reported here.

The first set of experiments used microcosms with and without sediment, and a low level of oil addition (1400 mg fresh ANS/L). Oil only or dispersant plus oil were added as treatments, in addition to the sediment treatments. For treatments including dispersant, the dispersant was added to the fresh ANS in these experiments at a rate of 1:10 (dispersant:oil). Dodecane, hexadecane, 2-methyl-naphthalene, phenanthrene, and pyrene were used as substrates.

Mass balance determinations also were performed in these first experiments for the substrates hexadecane, 2-methyl-naphthalene and phenanthrene in samples containing sediments. After collecting $^{14}CO_2$ from the samples, the vials were centrifuged (4000 x *g*) to pellet the sediment and the aqueous phase was recovered. Ten ml BH broth was then added to the vial, the vial shaken vigorously by hand for 30 seconds, then centrifuged again to pellet sediment. This added BH was retrieved and combined with the first aqueous fraction in pre-cleaned glass vials with Teflon-lined silicone septa. The remaining pellet was then extracted three times with 5 ml of a 1:1 (v/v) mixture of chloroform/MeOH, shaking vigorously by hand and centrifuging after each addition of solvent. The three 5-ml organic aliquots were then combined. Mass balances were calculated after measuring radioactivity by liquid scintillation counting (Beckman LSC2000 liquid scintillation counter; Beckman Instruments, Irvine, CA) in the aqueous (BH), organic (chloroform:MeOH) and gaseous (CO_2) phases.

The second set of radiorespirometry assays used a high level of oiling (4500 mg ANS/L) and fresh ANS crude or dispersant plus ANS, with and without sediment. Dispersant was added

at the rate of 1:10 (dispersant:oil), and hexadecane, 2-methyl-naphthalene, and phenanthrene were used as hydrocarbon substrates.

The third group of experiments substituted weathered ANS crude oil for the fresh oil used in the previous experiments, and a low oil addition rate was used. The oil was supplied by Battelle Ocean Sciences (Duxbury, MA), and had been artificially weathered using a modified method ASTM D86/82, resulting in losses of components boiling below 200°C (pers. comm., Scott Macomber, Battelle Ocean Sciences). No sediment was used in these assays, and treatments included addition of dispersant only, weathered oil only, or dispersed weathered oil. Two rates of dispersant addition were used, with a 1:10 (w/w) and a 1:20 (w/w) dispersant to oil ratio. Labeled hydrocarbon substrates added were the alkane dodecane and the PAH phenanthrene.

To assess the effects of nutrient addition on the selected consortium in our microcosm assay system, we performed a radiorespirometry assay using our standard 72-h inoculum from which had been removed residual nutrients and hydrocarbons remaining from the enrichment culture. This was to assure that we were assaying the metabolic potential of the microbial consortium absent any confounding influences associated with nutrient addition or growth *in vitro*. A 72-h culture was grown as described above, and the cells were harvested by centrifugation and washed by resuspending the pellet in sterile artificial seawater (SW; Crystal Sea® Marine Mix, Marine Enterprises International, Inc., Baltimore, MD). This was repeated three times, and the final pellet was either resuspended in SW or BH for non-amended and nutrient-amended treatments, respectively. Final volumes were identical with the original culture volumes to assure equivalent cell densities for the microcosm assays. No sediments were used in these experiments. For cultures suspended in SW, fresh ANS (low oiling rate) only or 1:10 dispersed ANS were used as treatments. For the nutrient-amended, BH suspensions, dispersant only, oil only (ANS, low oiling rate), 1:10 dispersed fresh ANS oil and 1:20 dispersed fresh ANS oil were the treatments. Dodecane and phenanthrene were used as hydrocarbon substrates.

Total C Mineralization

Mineralization of any carbon source also was assessed in some microcosms by gas chromatography (GC). Using either the standard enrichment inoculum (72-h culture) as described above, or freshly thawed inoculum diluted in sterile marine BH, we set up 40-ml

microcosms with 10 ml culture. To these microcosms we added: 1) (72-h culture) dispersant only, fresh ANS crude oil only, 1:10 (w/w) dispersed fresh ANS, or 1:20 (w/w) dispersed fresh ANS, and 2) (freshly-thawed, diluted inoculum) dispersant only, weathered ANS crude oil only, 1:10 dispersed weathered ANS or 1:20 dispersed weathered ANS. Treatments were run in triplicate, and CO_2 evolved was measured in the microcosm headspace every few days by a gas chromatograph equipped with a thermal conductivity detector (Shimadzu GC 14A gas chromatograph; Shimadzu Corp., Kyoto, Japan). Total CO_2 concentrations in the microcosms' headspace were converted to a C mass basis and cumulated to prepare time course plots of C mineralization.

Enumerations

Shake flask batch cultures using our inoculum were set up to track microbial growth in the presence of fresh ANS crude oil only (low oiling rate), dispersant only, or 1:10 (w/w) dispersed fresh ANS. Frozen inoculum was freshly thawed and added to 500-ml flasks containing 250 ml marine BH broth and one of the three treatments. Flasks were put on a shaker table and incubated for 8 days at 8°C. Aliquots of 100 µl were collected at *ca.* 24-h intervals for one week, fixed in formalin, and enumerated by direct count after staining as described previously (Lindstrom *et al*., 1998). After 8 days incubation, aliquots also were collected from the shake flasks for most-probable-number (MPN) enumeration of marine heterotrophs (using Marine Broth 2216; Atlas, 1993), crude oil emulsifiers (Sheen Screen technique; Brown and Braddock, 1990), hexadecane and phenanthrene degraders (Braddock *et al.*, 1997)

Oil Degradation

A two-month-long experiment was performed to assess the degree of degradation of crude oil in the presence of dispersant and/or our inoculum. Four 500-ml flasks were filled with 250 ml sterile marine BH broth and either kept sterile or inoculated with freshly thawed frozen consortium. To these flasks were added either *ca.* 90 mg fresh ANS crude oil or 90 mg 1:10 (w/w) dispersed fresh ANS crude oil. After shaking for two months at 8°C, the flasks' contents were transferred to pre-cleaned, 250-ml Boston round bottles (I-Chem Research, Hayward,CA), and 1.5 ml concentrated HCl was added to stop microbial activity. These samples, along with a sample of the fresh ANS crude oil originally added to the flasks, were analyzed using gas

chromatography/mass spectrometry (GC/MS) at University of Alaska Fairbanks's Water and Environmental Research Center. The GC/MS chromatograms were evaluated visually for evidence of oil degradation.

Results

Radiorespirometry

Assays conducted using fresh ANS crude oil in the low oiling regime responded differently to treatments depending on the radiolabeled substrate used. Pyrene, a four-ring PAH, was not used by our consortium over the course of the 168-h assay for this substrate (data not shown). When the 12-carbon, linear alkane dodecane was used as hydrocarbon substrate (Fig. 1A), no sediment or dispersant treatment effects were observed in the 96-h assay. Mineralization potentials for hexadecane, a 16-carbon alkane, were generally low but measurable (i.e., significantly higher than killed controls) for all treatments (Fig. 1B), and exhibited no treatment effects due to sediments. Addition of dispersed oil when sediments were absent, though, resulted in a diminished hexadecane mineralization potential ($p < 0.001$) compared to addition of oil only (Fig. 1B). Results for 2-methyl-naphthalene (Fig. 1C) were similar to those for dodecane, with neither sediment nor dispersant affecting our consortium's mineralization potential for this substrate. Phenanthrene mineralization potentials (Fig. 1D) were attenuated both by presence of sediment ($p = 0.07$) and by presence of dispersant ($p < 0.001$).

Mass balance calculations for hexadecane, 2-methyl-naphthalene and phenanthrene in vials with sediment yielded recoveries of greater than 65% for these substrates. Mean (± standard error, SE) recoveries were 76.2% (±6.1%) for hexadecane, 72% (±3%) for 2-methyl-naphthalene, and 67% (±4%) for phenanthrene. Aqueous solubilities of the substrates used appeared to dictate their partitioning in the microcosms, with mean (± SE) values for sediment-sorbed (i.e., organic solvent-extractable) fractions of 70% (±6%) for hexadecane, 14% (±2%) for 2-methyl-naphthalene, and 47% (±6%) for phenanthrene. Mean (± SE) aqueous phase values were 5% (±1%) for hexadecane, 12% (±1%) for 2-methyl-naphthalene, and 11% (±2%) for phenanthrene. Values for both sediment-sorbed and aqueous fractions were not significantly different between oil only and dispersed oil-treated microcosms (data not shown).

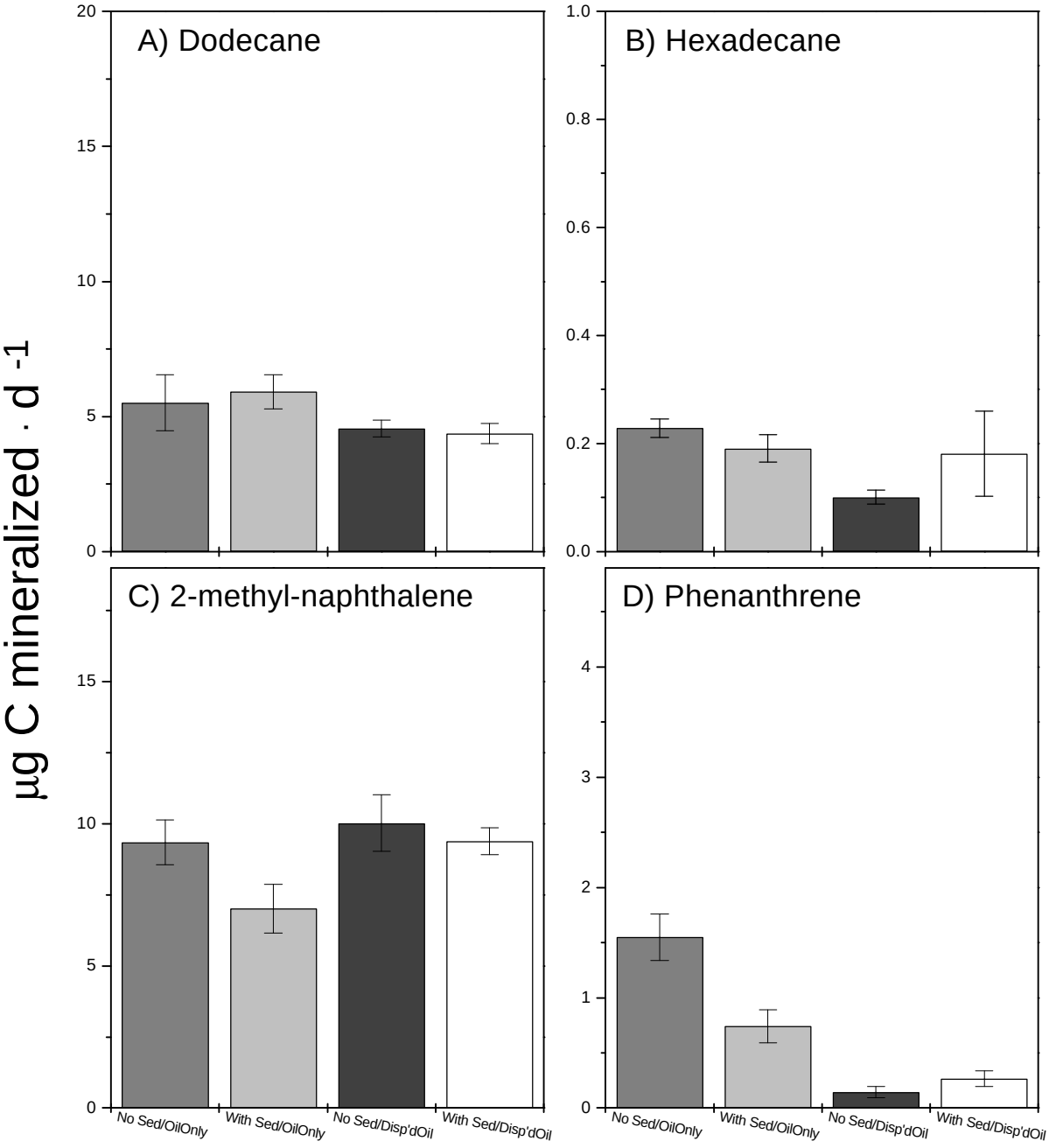

Figure 1. Mean hydrocarbon mineralization potentials under low oiling conditions using fresh ANS crude oil for A) dodecane, B) hexadecane, C) 2-methyl-naphthalene, and D) phenanthrene. Treatments were: No sediment with oil only added; Sediment with oil only added; No sediment with dispersed oil added; Sediment with dispersed oil added. Note scales are different for each substrate. Error bars are standard errors of the mean of five replicates.

When a high oiling level with fresh ANS crude oil was used, sediment presence resulted in no observable effect compared to the “no sediment” treatments for hexadecane (Fig. 2A), 2-methyl-naphthalene (Fig. 2B) or phenanthrene (Fig. 2C). Hexadecane mineralization potentials were again rather low but measurable, and were diminished in the presence of dispersed oil compared to addition of oil only ($p = 0.07$; Fig. 2A) when sediment was absent. Mineralization potentials for 2-methyl-naphthalene were unaffected by dispersed oil compared to oil only (Fig. 2B), but phenanthrene potentials were diminished in the presence of dispersed oil ($p < 0.001$ compared to oil only; Fig. 2C) when sediment was absent.

When weathered ANS crude oil was used in the assays, results were similar to those seen using the fresh ANS crude oil. In these assays dispersant alone and 1:20 dispersant:oil mixture were added to the oil only and 1:10 dispersed oil treatments. Sediment was not used in these assays as it was not generally found to have significant treatment effects in our test system. For dodecane (Fig. 3A), as with the previous assays, presence of dispersed oil in a 1:10 dispersant:oil ratio yielded results no different from those seen for oil only treatments; dispersant only and 1:20 dispersed oil treatments similarly did not show a difference. For the substrates hexadecane (Fig. 3B) and phenanthrene (Fig. 3C), however, presence of dispersant, whether alone or mixed with weathered oil at a 1:10 or 1:20 ratio, resulted in reduced mineralization potentials ($p < 0.001$ for each substrate’s dispersant treatment compared to oil only).

Washed cells from our consortium resuspended in artificial seawater (SW) or nutrient-amended marine Bushnell-Haas broth (BH) yielded similar results irrespective of nutrient amendment (Figures 4A and 4B). In other words, for the short duration of our radiorespirometry assays, nutrient addition did not affect the metabolic capabilities of our microbes. As with the other low oiling, fresh ANS experiment (Figures 1), dispersant presence had no effect on dodecane mineralization potentials (Figure 4A). Phenanthrene mineralization potentials in the presence of dispersant only or 1:10 dispersed oil (Figure 4B), however, were lower ($p < 0.001$) than that seen for the oil only treatment. No treatment effect was seen for the 1:20 dispersed oil treatment.

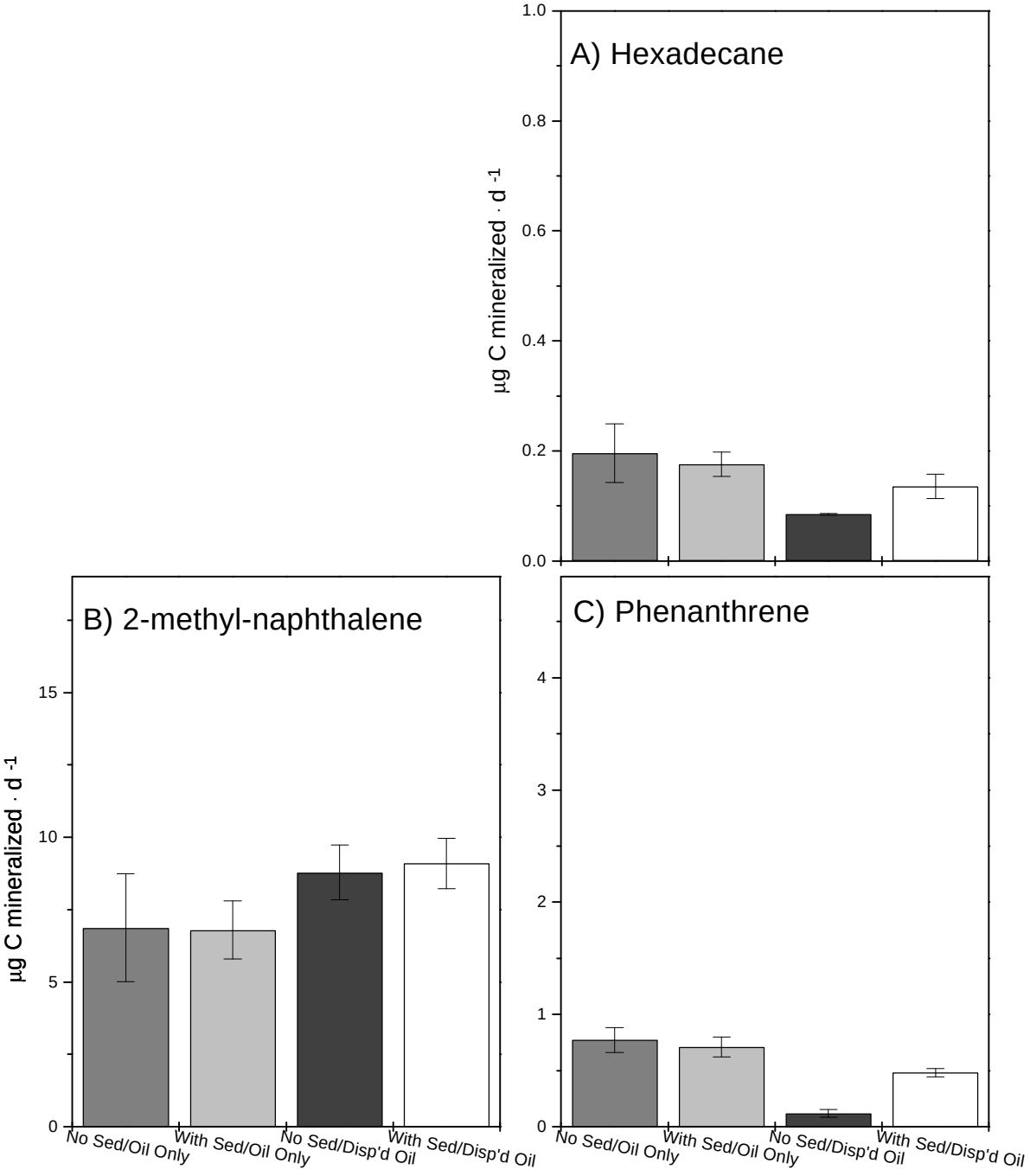

Figure 2. Mean hydrocarbon mineralization potentials under high oiling conditions using fresh ANS crude oil for A) hexadecane, B) 2-methyl-naphthalene, and C) phenanthrene. Treatments were: No sediment with oil only added; Sediment with oil only added; No sediment with dispersed oil added; Sediment with dispersed oil added. Note scales are different for each substrate. Error bars are standard errors of the mean of five replicates.

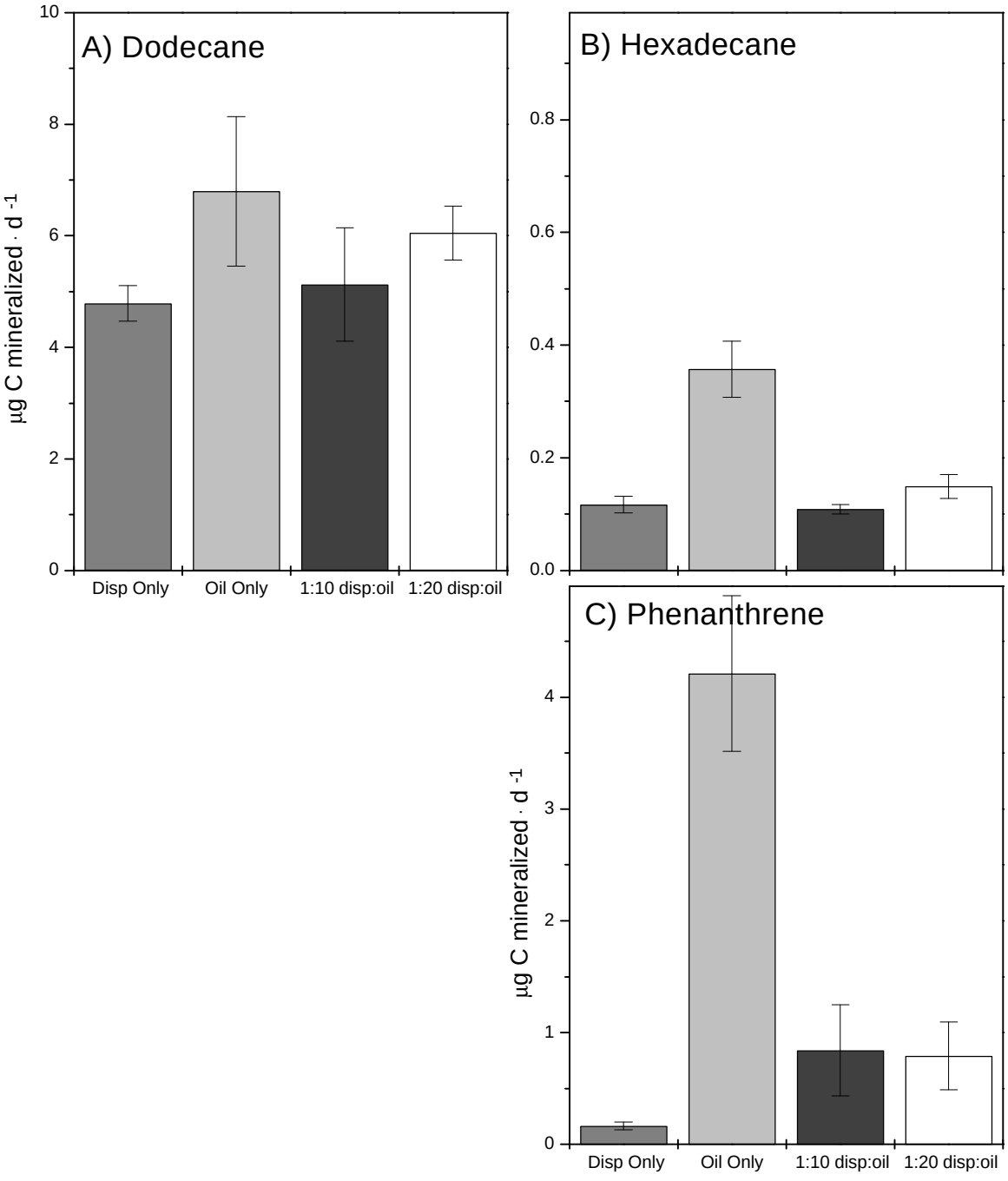

Figure 3. Mean hydrocarbon mineralization potentials under low oiling conditions using weathered ANS crude oil. A) hexadecane, B)2-methyl-naphthalene, and C) phenanthrene. No sediments were used. Treatments were: Dispersant only added; Oil only added; 1:10 dispersant:oil added; 1:20 dispersant:oil added. Note scales are different for each substrate. Error bars are standard errors of the mean of five replicates.

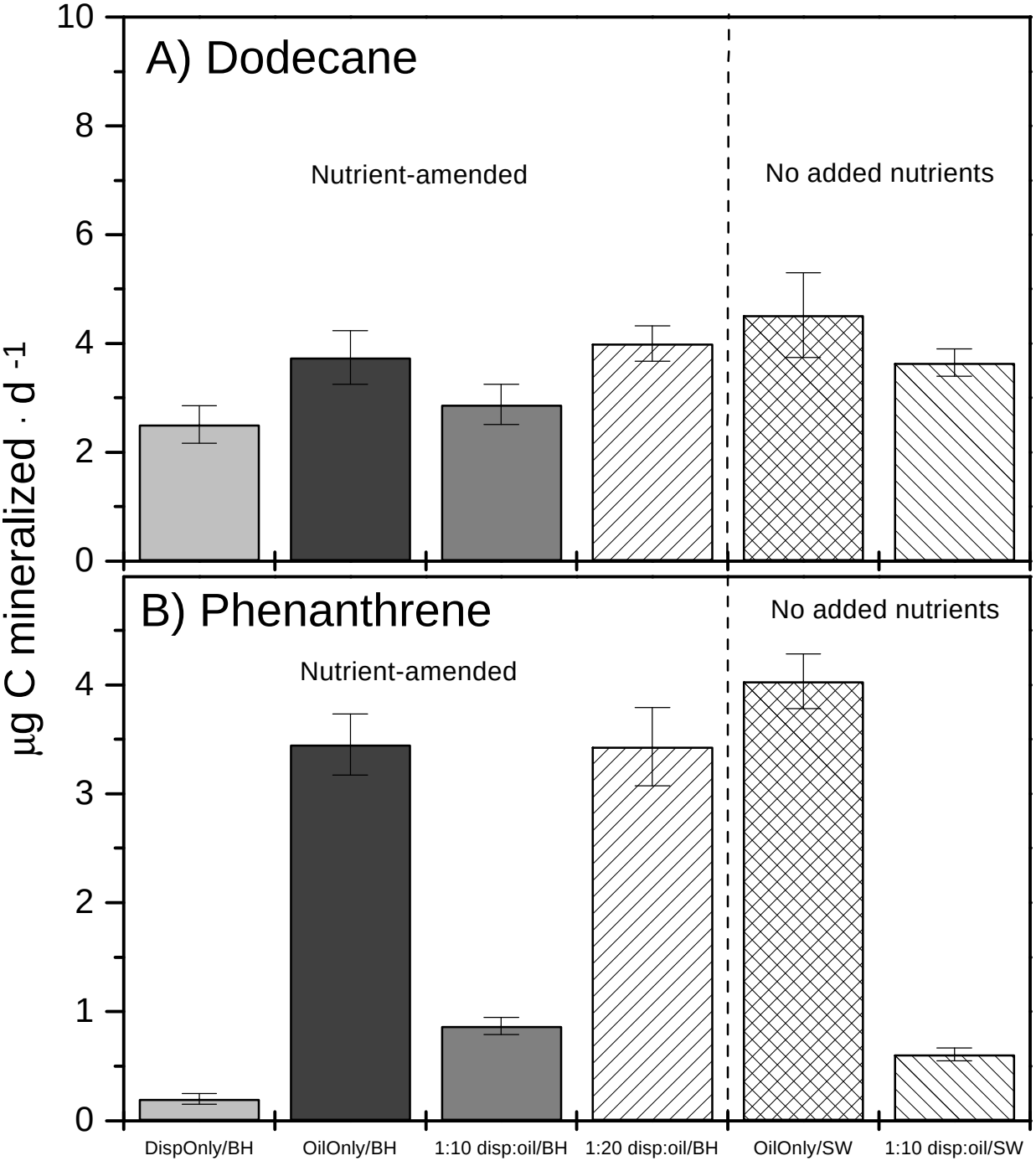

Figure 4. Mean hydrocarbon mineralization potentials for washed cells with and without added nutrients. A low oiling rate with fresh ANS crude oil was used. A) hexadecane, B) 2-methyl-naphthalene, and C) phenanthrene. No sediments were used. Treatments were: Dispersant only added; Oil only added; 1:10 dispersant:oil added; 1:20 dispersant:oil added. BH = nutrient-rich marine Bushnell-Haas broth; SW = artificial seawater. Note scales are different for each substrate. Error bars are standard errors of the mean of five replicates.

Total C Mineralization

Time course plots of C mineralized in microcosms containing either 72-h enrichment culture (Figure 5A) or freshly thawed inoculum (Figure 5B) showed similar results. In both cases, the microbial consortia respired more rapidly when offered dispersant only compared to respiration rates for oil only, or 1:10 and 1:20 dispersed oil. For the 72-h culture, absence of a lag period demonstrates that the microbes present in the microcosms were actively metabolizing organic substrates from the start of the experiment (Figure 5A). The 1:10 dispersant:oil mixture treatment was less active than the dispersant only treatment, which was more active than the 1:20 dispersant:oil treatment. All dispersant treatments for this experiment resulted in more actively respiring consortia than the fresh ANS oil only treatment.

There was a longer lag time observed for the freshly thawed consortium (Figure 5B) than for the 72-h culture (Figure 5A). This experiment used weathered ANS crude oil and, over the duration of the experiment, the dispersant only treatment showed the greatest respiration activity. Dispersed oil (1:10 or 1:20) or weathered oil only treatments were not different from each other over the course of this assay (Figure 5B).

Enumerations

Direct count enumeration data (Figure 6) for consortium shake flask batch cultures grown on dispersant, fresh ANS crude oil and a 1:10 (w/w) dispersant:fresh ANS mixture indicate that all three treatments supported microbial growth and that this growth was exponential from Day One through five days. Little difference in growth due to treatment was observed. Most probable number (MPN) enumerations from samples collected on Day 8 (Figure 7) demonstrate that all three substrate types (i.e., dispersant only, oil only or dispersed oil) could support large populations of marine heterotrophs. Sheen Screen MPN enumerations indicated that oil emulsifiers also were abundant (Figure 7), with dispersant only and oil only treatments yielding more than 10^5 cells per ml culture; data for the dispersed oil treatment were not recoverable from that enumeration assay. Substrate-specific enumerations for hexadecane and phenanthrene (Figure 7) suggested that few hexadecane degraders were present in these cultures at 8 days, but phenanthrene degraders were 10- to 1000-fold more abundant, depending on the treatment. Significantly higher populations metabolizing both substrates were seen in flasks with added oil (either with or without dispersant).

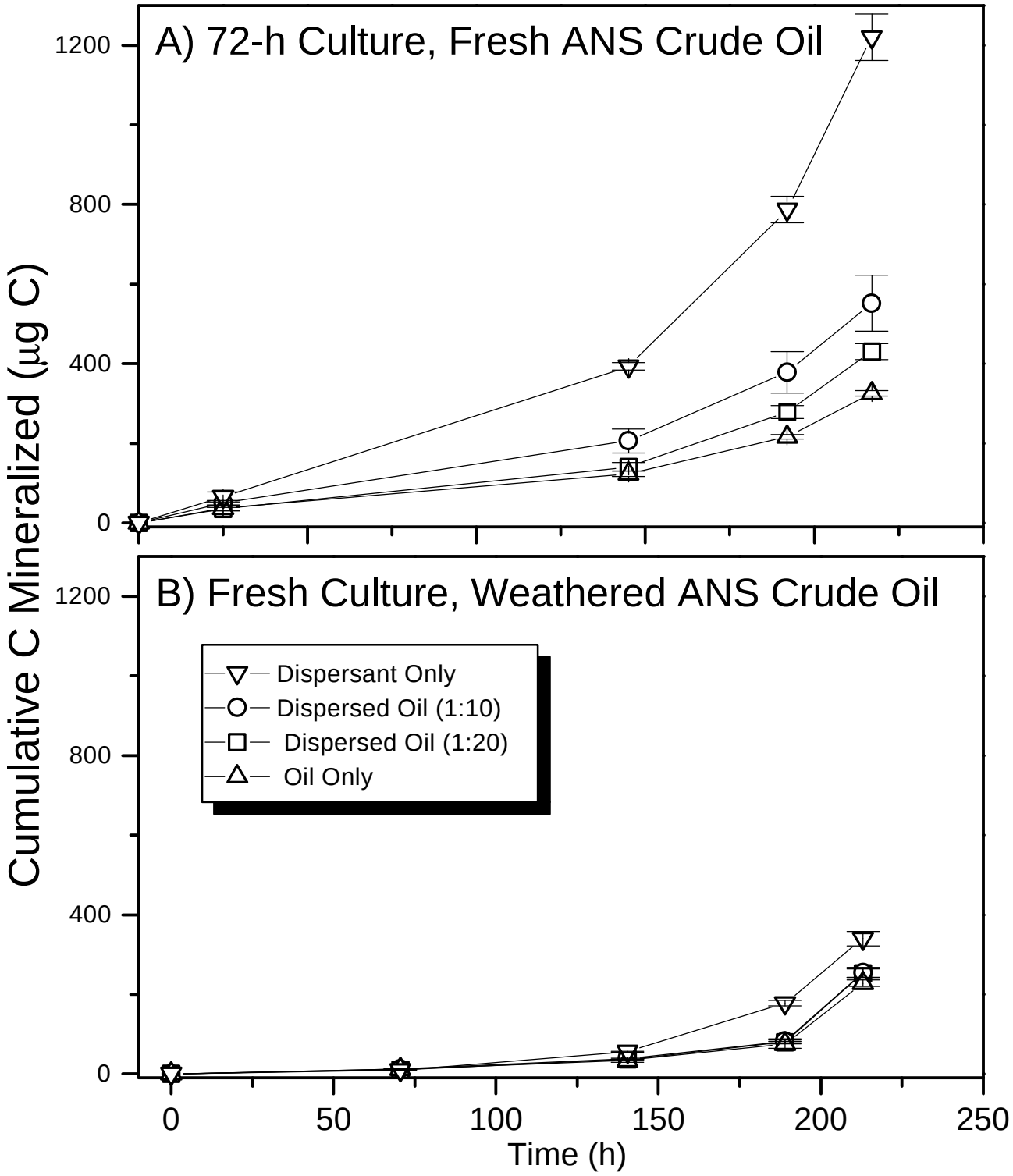

Figure 5. Mean cumulative total C mineralized over time for A) 72-h culture of consortium, and B) freshly thawed consortium. Treatments for the 72-h culture were Dispersant Only, 1:10 dispersant:fresh ANS oil mixture, 1:20 dispersant:fresh ANS oil mixture, and fresh ANS oil only added. For the freshly thawed consortium, the treatments were the same except that weathered ANS crude oil was used instead of fresh ANS crude oil. Error bars are standard errors of the mean of triplicate analyses.

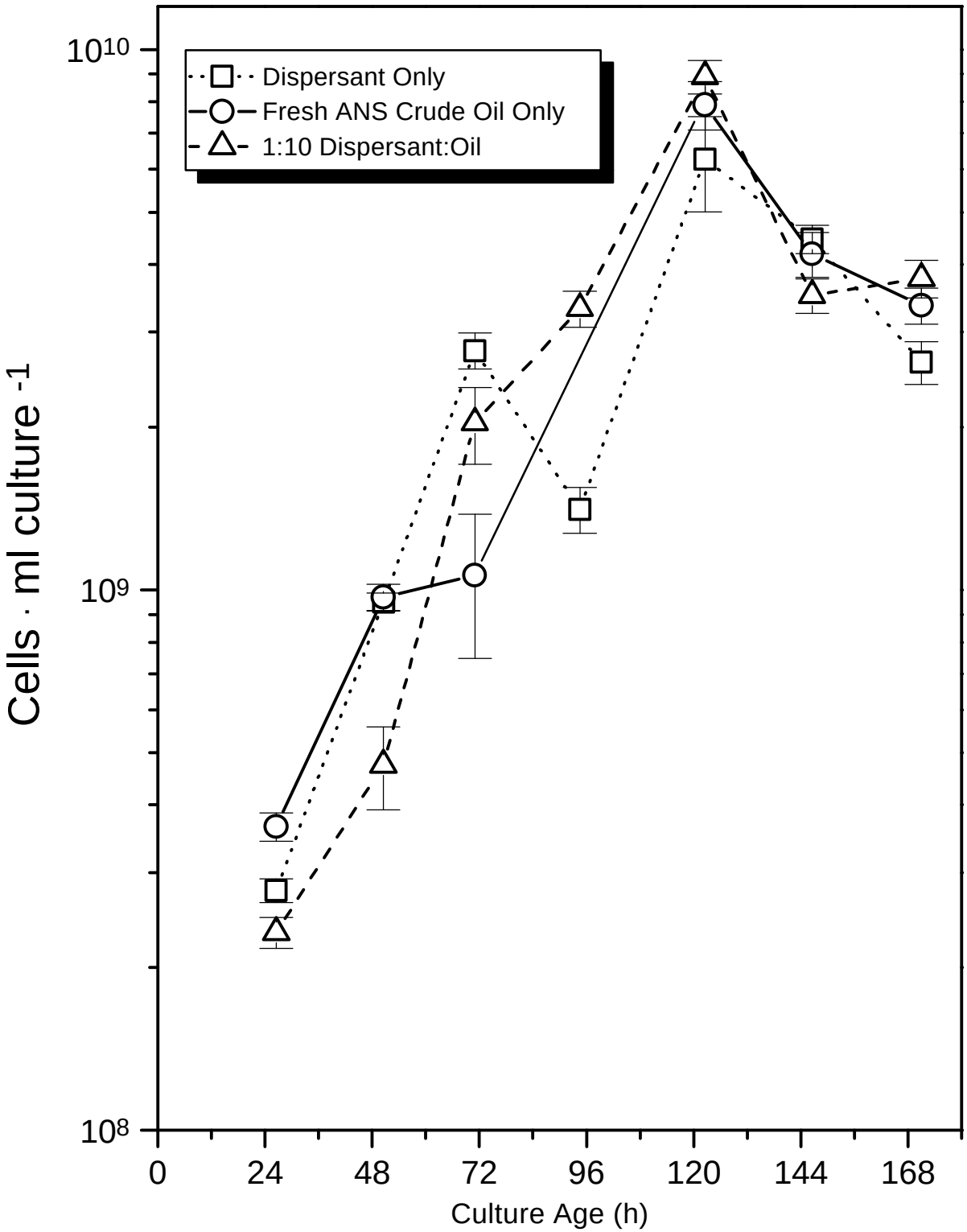

Figure 6. Direct count enumerations for consortium grown in marine BH broth with only dispersant added, only fresh ANS crude oil added, and 1:10 dispersant:fresh ANS oil added. Data are mean values (error bars are standard errors of the mean) of counts determined for 10 observations of each culture aliquot at a given time. Vertical scale is logarithmic, and all three cultures were growing exponentially through 120 h.

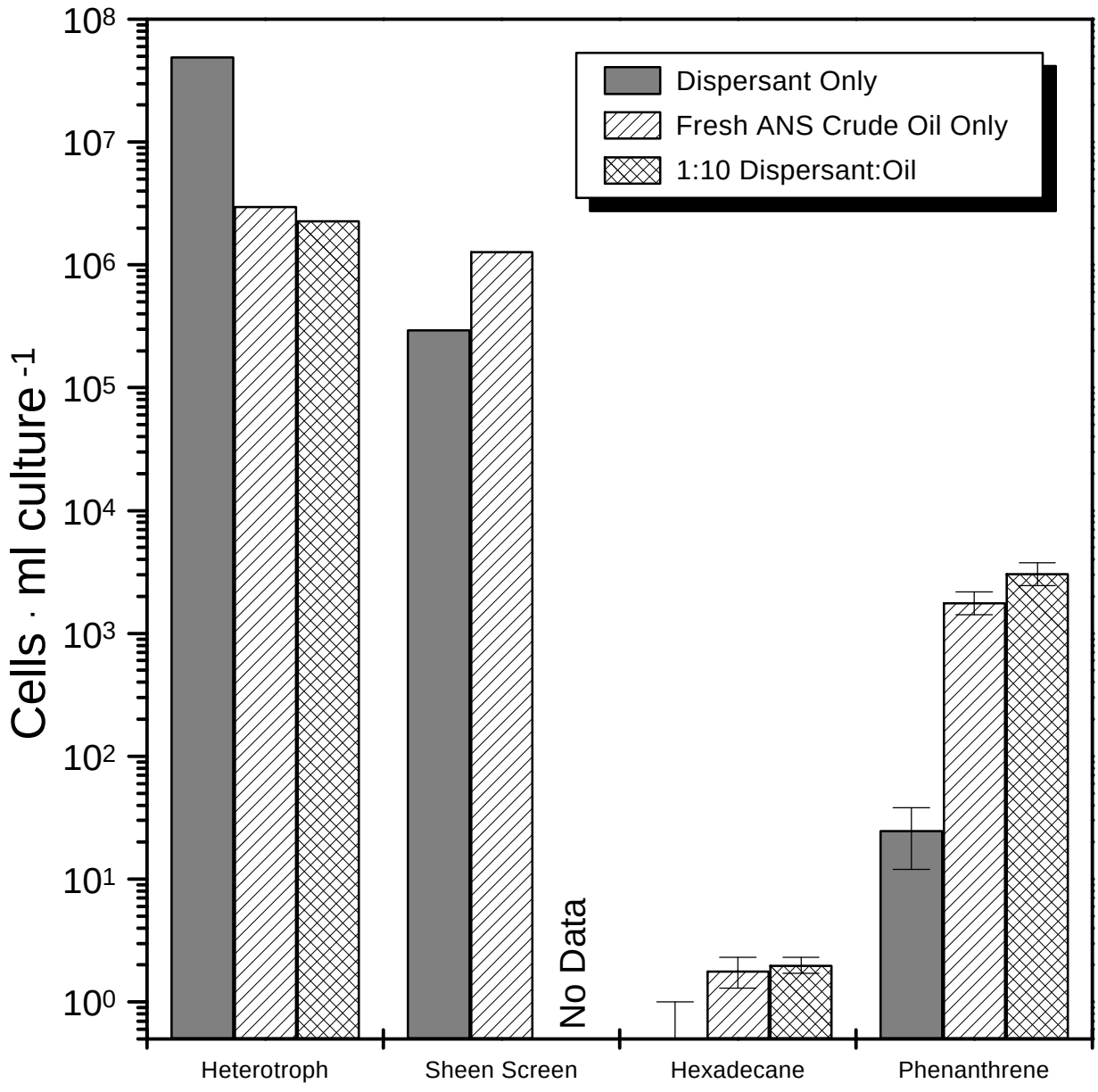

Figure 7. Shake flask batch culture most-probable-number (MPN) enumerations at 8 days. Microbial consortium was grown on dispersant only, fresh ANS crude oil only, or a 1:10 (w/w) mixture of dispersant and fresh ANS oil. Sheen Screen MPN data are missing for the 1:10 dispersant:oil treatment. Dispersant, oil and dispersed oil all supported large populations of heterotrophs, and dispersant and oil also supported significant populations of oil emulsifiers (Sheen Screen assay). Smaller populations of hexadecane and phenanthrene degraders (means and standard errors of triplicates shown) were observed with the growth substrates offered.

Oil Degradation

Qualitative analysis of residual oil remaining after two months (Figure 8) indicated that oil degradation was much more extensive in inoculated than in sterile treatments. The data also suggest that dispersed oil lost more of the linear alkane fraction (discrete peaks in chromatogram) than oil without dispersant.

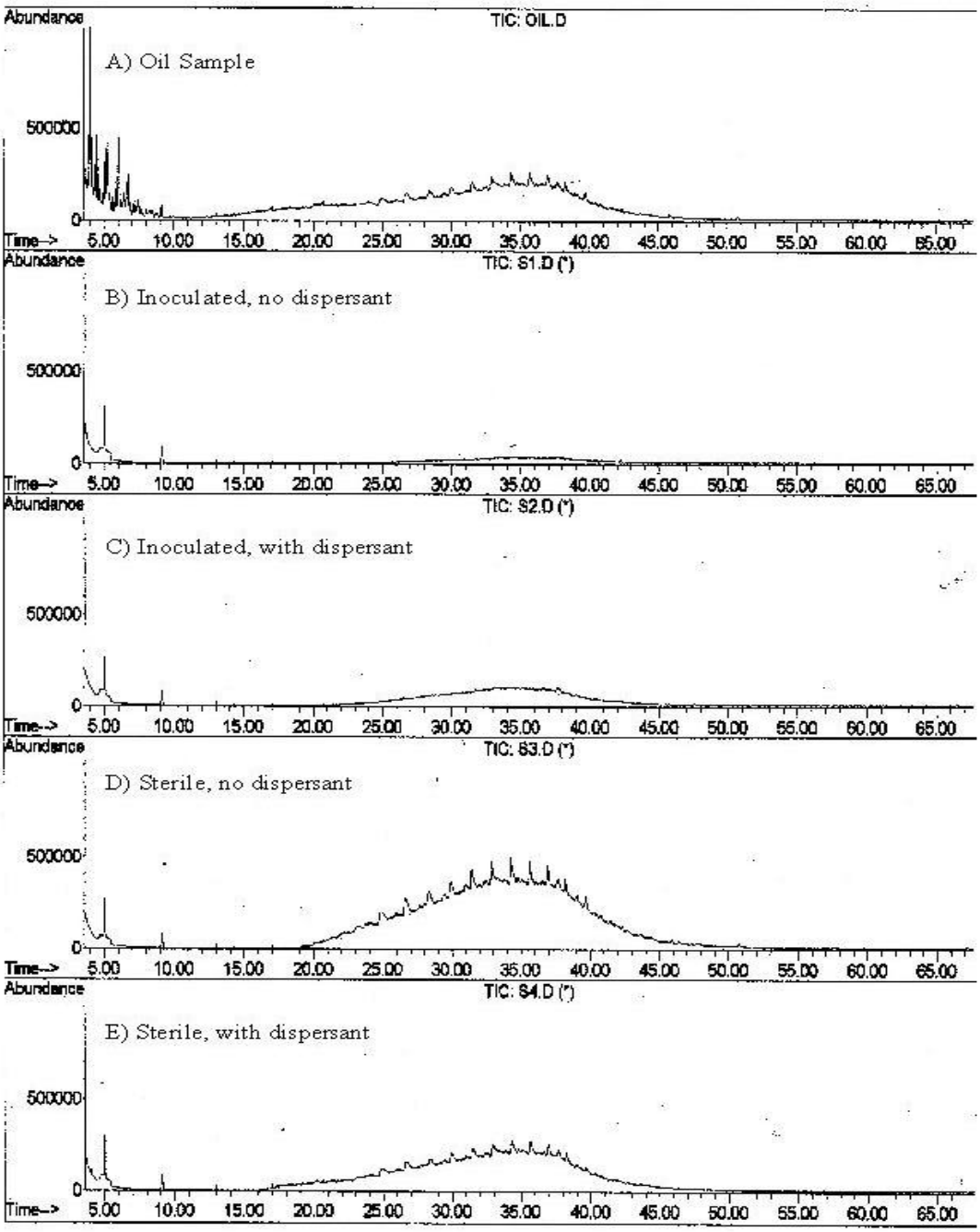

Figure 8. Chromatograms of fresh ANS crude oil and material recovered from two-month-old flask batch cultures. A) oil used to spike flasks; B) material from flask containing microbial consortium and fresh ANS crude oil; C) material from flask containing consortium and dispersed fresh ANS crude oil; D) material from sterile flask containing fresh ANS crude oil; E) material from sterile flask containing dispersed ANS crude oil.

Discussion

The only available study regarding the effects of Corexit 9500 on biodegradation of oil under simulated marine conditions (Swannell *et al.,* 1997) found that addition of dispersant stimulated biodegradation of oil in the water column. Our findings are consistent with the previous study's results, though our methodology has yielded data suggesting a somewhat different conclusion. The conclusions by Swannell et al (1997) were based on the observation that dispersed oil droplets in microcosms were colonized by microbes, leading to elevated numbers and decreased size of the droplets. Further, they found that colonized oil droplets were stabilized in the aqueous medium, suggesting that the microbes limited the tendency of the droplets to re-coalesce. Most importantly, they found that dispersant also led to higher numbers of hydrocarbon-degrading microbes and elevation of CO_2 from the microcosms, particularly with the addition of nutrients (nitrate). Our results corroborate some of the findings of the previous Corexit 9500 study, but the resolution of the radiorespirometric assays provides data suggesting that dispersant-enhanced oil mineralization may be restricted to particular components of the crude oil.

Our study examined the effects of Corexit 9500 and sediment on microbial mineralization of specific aliphatic and aromatic hydrocarbons found in crude oil. Additionally, we measured gross mineralization of crude oil, dispersed crude oil and dispersant by our marine consortium in the absence of sediment. With the exception of phenanthrene in the low oiling experiment (see Figure 1D), sediment had no statistically significant effect on hydrocarbon mineralization potentials in our microcosms. The sediment we used was an intertidal, sandy marine sediment with a low organic matter content. Several studies have suggested that clays and organic matter are the most important aspects affecting marine sediment sorption of hydrocarbons (see NRC, 1985, Chapter 4 'Fates', for a review of hydrocarbon sorption and sedimentation). Karickhoff *et al.* (1979) found that the sand fraction of marine sediments was a less effective adsorbent than the silt or clay fractions, and differences between the silt and clay fractions were due to their respective organic matter contents. Thus our choice of sediment type may well have affected the strength of the hydrocarbon-sediment interaction, resulting in our hydrocarbon substrates being weakly bound and readily available to our marine consortium. Despite the results of our mass

balance assessment that indicated that the less soluble substrates were associated with microcosm sediments, this association had little effect on the hydrocarbon mineralization potentials for our consortium. Thus, sediment was omitted from all experiments following the low and high oiling experiments.

For the assays not including sediment, treatment effects associated with dispersant presence varied with respect to substrate (Figures 1 – 4), but these effects were consistent whether fresh ANS (low or high oiling rates) or weathered ANS was used in the microcosms. For the aliphatic hydrocarbons, dodecane mineralization potentials were not affected by the presence of dispersed oil (Figures 1A, 3A and 4A) or dispersant only (Figure 4A). Hexadecane potentials, on the other hand, were attenuated both by the presence of dispersed oil (Figures 1B, 2B and 3B) and dispersant only (Figure 3B). For the aromatic hydrocarbons, 2-methyl-naphthalene mineralization potentials (Figures 1C and 2B) with and without dispersed oil were not significantly different from each other. In contrast, phenanthrene potentials were significantly diminished when dispersant was present, whether in fresh or weathered dispersed oil (Figures1C, 2C and 3C) or as dispersant alone in the microcosm (Figures 3C and 4B). Differences in solubility behavior among these substrates may explain the radiorespirometry data. Dodecane, a 12-carbon, volatile liquid hydrocarbon (NRC, 1985) is substantially more soluble in aqueous media than is the 16-carbon hexadecane. Likewise, 2-methyl-naphthalene, a two-ring PAH, is much more soluble than is phenanthrene, a three-ring PAH (NRC, 1985). Thus the results of the radiorespirometry assays suggest that our marine consortium was metabolizing the soluble carbon substrates in preference to those that were insoluble. Since at least some components of the dispersant represent substrates for growth (Swannell 1997), preferential use of soluble components of the dispersant may have resulted in lower mineralization potentials for hexadecane and phenanthrene. The potentials for the more soluble substrates dodecane and 2-methyl-naphthalene, on the other hand, were not diminished by dispersant, suggesting that relatively soluble substrates are not affected by dispersant addition.

Nutrient addition had no effect on the radiorespirometry assays, as evidenced by the similar responses of nutrient-amended and non-amended washed cells to oil and dispersed oil addition (Figure 4). Swannell et al. (1997) found that nutrient addition increased carbon mineralization rates in their microcosms, but our assays differed from the system used in their study. We used log-phase cultures (see below) of our consortium in a short-term assay designed to measure the

metabolic potential of the extant community of microbes in the microcosm. Our assay is of short duration to avoid growth of, and acclimation to the test substrate by, populations within the community being assessed (Brown et al. 1991). The authors of the other study (Swannell *et al.*, 1997) designed their experiments to examine growth of microbes in the presence of dispersed oil and nutrients, and tested the response of defined or natural marine consortia to nutrient addition. Thus, while we measured the short-term metabolic capabilities of a large, log-phase culture in conditions of balanced growth, the other investigators examined the effects of nutrients on growth of a small population over the course of up to 28 days. Our results show that our consortium exhibited an inhibition of phenanthrene mineralization due to dispersant presence irrespective of nutrient amendments. Thus we are confident that our other radiorespirometry assays (Figures 1 to 3) were not biased by amending the microcosms with nutrients.

Evidence for preferential use of dispersant over less soluble hydrocarbon components is provided by the data presented in Figure 5. When dispersant alone was added to either the 72-h culture (Figure 5A) or a freshly thawed frozen consortium (Figure 5B), mineralization of organic carbon was significantly higher than dispersed oil or oil only. Indeed, for the 72-hour culture (Figure 5A), a dose-response relationship was observed, with cumulative C mineralization higher for the dispersed oil containing a higher concentration of dispersant (1:10 dispersant:oil ratio). This dose-response relationship was not seen for the experiment using weathered oil (Figure 5B). This difference may be explained by higher concentrations of dispersant increasing the bulk solubility of the fresh ANS oil more than low dispersant concentrations, and a concomitant increase in solution of easily metabolized labile hydrocarbons (absent from the weathered crude oil), or by preferential use of the relatively more abundant dispersant in the 1:10 versus the 1:20 dispersed oil.

Growth in batch culture of our marine consortium on fresh ANS, dispersed ANS and dispersant only (Figures 6 and 7) exhibited similar patterns. All three substrate combinations yielded exponential growth of our consortium through 120 hours (Figure 6; note logarithmic scale), indicating that fresh ANS crude and dispersant represent similarly labile sources of carbon for heterotrophic growth. These data also indicate that the cells used in our radiorespirometry assays (72-h batch cultures) were growing logarithmically when added to the microcosms. The data in Figure 6, however, are based on direct microscopic counts of microbes and do not discriminate cells according to metabolic type. The same batch cultures enumerated

to provide time-course growth data for Figure 6 also were examined by culture-based techniques after about a week to determine relative abundances of specific metabolic types of microbes. These data (Figure 7) indicate that Corexit 9500 (Dispersant Only) supported the growth of a large population of heterotrophs and hydrocarbon degraders (Sheen Screen assay), a finding in concert with those of Swannell et al. (1997). Dispersant alone, dispersed oil or oil alone, did not induce much growth of hexadecane degraders (Figure 7), and this is consistent with the hexadecane radiorespirometry activity data (Figure 1 for oil only and dispersed oil, Figure 3 for dispersant only, oil only and dispersed oil) which was generally low overall. Phenanthrene degraders were much lower in the dispersant only batch culture (Figure 7), but about 100-fold higher in oil only or dispersed oil cultures. No significant difference was seen between the population responses for dispersed oil and oil only treatments (Figure 7), though phenanthrene mineralization activities were affected by dispersant (Figures 1D, 2C, 3C, and 4B). The different nature of the MPN and radiorespirometry assays suggests an explanation for these apparently contradictory results. The MPN assay provides phenanthrene as sole carbon source in the enumeration microplate, so any organisms able to grow in that microplate environment on phenanthrene should be enumerated. In the radiorespirometry assays, though, several soluble and labile carbon sources (e.g., dispersant, soluble crude oil components) were available to the consortium community and preferential use of substrates other than the ^{14}C-labeled phenanthrene was possible.

Examination of the gas chromatographs (Figure 8) indicates that the presence of microbes was necessary for oil degradation in the batch culture flasks, as the sterile oil only and dispersed oil flasks show little or no loss of the linear alkane fraction (discrete peaks apparent in chromatogram). While this analysis was not quantitative, it appears that, in flasks containing the microbial consortium, dispersant addition resulted in greater loss of some components of the oil compared to the oil only treatment. Our results comparing sterile with inoculated cultures are consistent with the findings of Swannell *et al.* (1997) who observed that nutrient-amended and non-amended cultures degraded much more of the alkane fraction than killed controls over the course of their microcosm studies.

We stated at the beginning of this Discussion that our findings corroborated some of the results reported by Swannell *et al.* (1997), but that our conclusions were somewhat different owing to the different natures of our respective test systems. It is important to note that the

previous study by Swannell *et al.* (1997) examined the effects of nutrients on the growth and mineralization activity of defined and natural microbial consortia in systems with dispersed oil. They performed no microbial activity experiments (i.e., those measuring carbon mineralization) with microcosms containing oil only or dispersant only. They did assess the growth of microbes in microcosms containing dispersant only and found that growth of hydrocarbon degraders and "dispersant degraders" occurred, and more so when additional nutrients were added. Our experiments provide information on the response of a test microbial consortium to dispersant versus no dispersant (irrespective of nutrients) with respect to population growth, carbon mineralization potential, and substrate-specific mineralization potential. We found that adding dispersant indeed led to elevated production of CO_2, but we cannot directly compare our data with the other study as the other authors reported no data regarding responses to oil without dispersant, or comparing dispersed oil with dispersant only or oil only.

Our data, evaluated as a whole, suggest that elevated mineralization activity in the presence of dispersant may be due to the presence of dispersant *per se*, and perhaps not to increased mineralization of all components of the crude oil present. It is clear from our study that the hydrocarbon-degrading marine consortium we used was able to grow on dispersant alone as well as on dispersed oil (see Figures 5, 6 and 7). Swannell *et al.* (1997) found that increasing nutrients in dispersed oil microcosms led to elevated carbon mineralization, a finding consistent with observations made by others (e.g., Bartha and Atlas, 1987). We also found high levels of carbon mineralization for dispersed oil, but the highest mineralization potentials (non-labeled carbon) were seen in the dispersant only assays (see Figure 5). Radiorespirometry data suggest that addition of dispersant may inhibit mineralization of relatively insoluble substrates (e.g., hexadecane and phenanthrene; see Figures 1, 2 and 3), perhaps due to microbial preference for the more bioavailable substrates. Our results do not contradict the findings of the only other report of Corexit 9500's effects on hydrocarbon mineralization, but they do suggest that more study is needed to evaluate how this dispersant affects biodegradation of specific fractions of crude oil. Clearly, dispersing oil will lead to increasing the surface area of contact between the aqueous and oil phases in a marine oil spill, thus providing a greater opportunity for microbial attack on the spilled oil. This should be measurable as an increase in carbon mineralization (CO_2 evolution) and numbers of hydrocarbon degraders, as found in the other study. But it is evident that some components of crude oil are more soluble and metabolically labile than others, and

assessing dispersant effects on microbial activity by total CO_2 measurements and total hydrocarbon degrader assays alone provides incomplete data. We found that dispersant addition to oil increased total CO_2 evolution from our defined consortium, but not as much as dispersant alone (Figure 5A), and hydrocarbon degraders were elevated much more than hexadecane degraders or phenanthrene degraders (Figure 7).

Conclusions

Our data suggest that addition of dispersant to oil increases total carbon mineralized, and numbers of hydrocarbon degraders. This suggests that dispersant increases oil biodegradation, but total C mineralization data and hydrocarbon degrader data are together insufficient to evaluate whether this observed increase in oil degradation is consistent across chemical classes of compounds contained in the oil. Our data indicate that dispersant may inhibit biodegradation of some components of the crude oil. At this point no data currently exist allowing evaluation of the effects of Corexit 9500 on biodegradation of the more acutely and chronically toxic components of crude oil. Following dispersant use, if the residual oil is selectively enriched in components of greater toxicity than those components biodegraded, the toxicity of the resulting oil residue (on an oil mass basis) may be increased.

We suggest that further studies be performed to evaluate the effects of dispersant on oil degradation in natural marine microbial consortia. Optimally, elements of the Swannell *et al.* (1997) study should be combined with the higher resolution offered by the assays used in our study to more closely evaluate Corexit 9500's effects on biodegradation of specific components of spilled crude oil. Replicate, 15 L, long-term growth microcosms (as used in the other study) containing adequate nutrients with dispersant only, oil only, or dispersed oil (as used in our study) should be employed. Total carbon mineralization should be monitored in the microcosms (as before), and this measurement should be supplemented by periodically determining numbers of total, heterotrophic, and specific hydrocarbon-metabolizing populations and assessing short-term mineralization potentials for specific hydrocarbon components of crude oil. Additionally, more detailed quantitative chemical analyses, including analyses for PAH, should be performed. While an expensive proposition, only this level of analysis will allow for a detailed evaluation of the effects of Corexit 9500 on crude oil biodegradation, and whether using this dispersant will decrease or increase the toxicity of oil spilled in a marine environment.

Acknowledgments

This project was funded by a grant from the Alaska Department of Environmental Conservation, Division of Spill Prevention and Response. We thank Kathleen Gannon and Huan Luong for their assistance in analyzing samples.

Literature Cited

Atlas, R.M. 1993. *Handbook of microbiological media*. CRC Press, Boca Raton, FL.

Bartha, R. and R.M Atlas. 1987. Transport and transformation of petroleum: biological processes, IN *Long-Term Environmental Effects of Offshore Oil and Gas Development* (D.F. Boesch and N.N. Rabalais, eds.), pages 287-341. Elsevier Applied Science Publishers Ltd., London.

Braddock, J.F., M.L. Ruth, P.H. Catterall, J.L. Walworth, and K.A. McCarthy. 1997. Enhancement and inhibition of microbial activity in hydrocarbon-contaminated arctic soils: implications for nutrient-amended bioremediation. *Environ. Sci. Technol.* 31:2078-2084.

Braddock, J. F. and Z. D. Richter. 1997. Microbial degradation of aromatic hydrocarbons in marine sediments. *University of Alaska Coastal Marine Institute Annual Report No. 3 FY1996, OCS Study MMS 97-0001*.

Brown, E. J. and J. F. Braddock. 1990. Sheen Screen, a miniaturized most probable number method for enumeration of oil-degrading microorganisms. *Appl. Environ. Microbiol.* 56: 3895-3896.

Brown, E. J., S. M. Resnick, C. Rebstock, H. V. Luong and J. Lindstrom. 1991. UAF radiorespirometric protocol for assessing hydrocarbon mineralization potential in environmental samples. *Biodegradation* 2: 121-127.

Bruheim, P., H. Bredholt and K. Eimhjellen. 1997. Bacterial degradation of emulsified crude oil and the effect of various surfactants. *Can. J. Microbiol*. 43: 17-22.

Foght, J.M. and D.W.S. Westlake. 1982. Effect of the dispersant Corexit 9527 on the microbial degradation of Prudhoe Bay oil. *Can. J. Microbiol*. 28: 117-122.

Harvey, S., I. Elashvili, J.J. Valdes, D. Kamely and A.M. Chakrabarty. 1990. Enhanced removal of Exxon Valdez spilled oil from Alaskan gravel by a microbial surfactant. *Bio/Technology* 8:228-230.

Karickhoff, S.W., D.S. Brown, and T.A. Scott. 1979. Sorption of hydrophobic pollutants on natural sediments. *Water Res.* 13:241-248.

Lindstrom, J.E., R.P.Barry, and J.F. Braddock. 1998. Microbial community analysis: a kinetic approach to constructing potential C source utilization patterns. *Soil Biol. Biochem.* 30:231-239.

NRC (National Research Council). 1985. *Oil In The Sea: Inputs, Fates, and Effects*. National Academy Press, Washington, D.C.

Rhoton, S.L., R.A. Perkins, J. Lindstrom, J. Braddock, and C. Behr-Andres. 1998. Toxicity of dispersants and dispersed oil to Alaskan marine organisms. *The 21st Arctic and Marine Oilspill Program Technical Seminar*, Edmonton, Alberta, June.

Rhoton, S.L., R.A. Perkins, Z.D. Richter, C. Behr-Andres, J.E. Lindstrom and J.F. Braddock. 1999. Toxicity of dispersants and dispersed oil to an Alaskan marine organism. *International Oil Spill Conference*, Seattle, Washington, March.

Rittmann, B.E. and N.M. Johnson. 1989. Rapid biological clean-up of soils contaminated with lubricating oil. *Wat. Sci Tech.* 21:209-219.

Swannell, R.P.J., F. Daniel, B.C. Croft, M.A. Engelhardt, S. Wilson, D.J. Mitchell, and T. Lunel. 1997. Influence of physical and chemical dispersion on the biodegradation of oil under simulated marine conditions. *Proceedings of the 20th Arctic and Marine Oilspill Program Technical Seminar*, Vancouver, British Columbia, Canada.

Tsomides, H.J., J.B. Hughes, J.M. Thomas and C.H. Ward. 1995. Effect of surfactant addition on phenanthrene biodegradation in sediments. *Environ. Toxicol. Chem.* 14: 953-959.

Tumeo, M., J. Braddock, T. Venator, S. Rog and D. Owens. 1994. Effectiveness of a biosurfactant in removing weathered crude oil from subsurface beach material. *Spill Sci. Technol. Bull.* 1:53-59.

Wolfe, D.A., M.J. Hameedi, J.A. Galt, G. Watabayashi, J. Short, C. O'Clair, S. Rice, J. Michel, J.R. Payne, J. Braddock, S. Hanna, and D. Sale. 1994. The fate of the oil spilled from the Exxon Valdez. *Environ. Sci. Technol.* 28:561A-568A.

APPENDIX

Results from all respiration assays

TOTAL C MINERALIZATION DATA, 72-h CULTURE, FRESH ANS CRUDE OIL

μg C mineralized at each sampling

Treatment	***25h***	***145h***	***192h***	***217h***
1:20 Disp:oil	37.9	94.8	149.1	158.0
1:20 Disp:oil	29.0	99.0	120.3	144.2
1:20 Disp:oil	38.4	124.3	143.4	154.7
mean value	35.1	106.0	137.6	152.3
Std error	3.1	9.2	8.8	4.1
1:10 Disp:oil	ND	117.0	147.4	152.8
1:10 Disp:oil	56.9	128.5	152.8	157.9
1:10 Disp:oil	44.8	220.9	217.1	208.3
mean value	50.8	155.5	172.4	173.0
Std error	6.0	32.9	22.4	17.7
Oil only	29.9	91.5	100.8	113.2
Oil only	31.3	80.3	93.9	106.1
Oil only	51.0	84.9	86.0	107.3
mean value	37.4	85.6	93.5	108.9
Std error	6.8	3.2	4.3	2.2
Disp only	88.9	293.6	345.7	380.6
Disp only	60.5	325.7	404.1	454.7
Disp only	46.4	365.1	431.7	464.1
mean value	65.3	328.2	393.9	433.1
Std error	12.5	20.7	25.4	26.4

μg C mineralized, cumulative

Treatment	***25h***	***145h***	***192h***	***217h***
1:20 Disp:oil	37.9	132.7	281.8	439.8
1:20 Disp:oil	29.0	128.0	248.3	392.5
1:20 Disp:oil	38.4	162.7	306.1	460.8
mean value	35.1	141.1	278.7	431.0
Std error	3.1	10.9	16.8	20.2
1:10 Disp:oil	ND	167.8	315.2	468.1
1:10 Disp:oil	56.9	185.3	338.1	496.0
1:10 Disp:oil	44.8	265.6	482.8	691.0
mean value	50.8	206.3	378.7	551.7
Std error	6.0	30.1	52.4	70.1
Oil only	29.9	121.4	222.2	335.4
Oil only	31.3	111.6	205.5	311.6
Oil only	51.0	135.9	221.9	329.2
mean value	37.4	123.0	216.5	325.4
Std error	6.8	7.1	5.5	7.1
Disp only	88.9	382.5	728.2	1108.8
Disp only	60.5	386.2	790.3	1245.0
Disp only	46.4	411.6	843.3	1307.4
mean value	65.3	393.4	787.3	1220.4
Std error	12.5	9.1	33.3	58.6

TOTAL C MINERALIZATION DATA, FRESH CULTURE, WEATHERED ANS CRUDE OIL

µg C mineralized at each sampling

Treatment	*70.5 h*	*140.5 h*	*189 h*	*213 h*	*308 h*	*334.5 h*	*380 h*
1:20 Disp:oil	11.6	24.2	41.7	152.8	1328.5	1254.0	1102.9
1:20 Disp:oil	11.0	24.1	44.5	184.7	1065.0	1007.9	930.8
1:20 Disp:oil	10.1	27.1	45.1	182.0	1667.0	878.0	880.6
Mean value	10.9	25.1	43.8	173.2	1353.5	1046.7	971.4
Std Error	0.4	1.0	1.1	10.2	174.2	110.2	67.3
1:10 Disp:oil	10.2	28.9	50.2	185.0	1299.6	879.5	858.2
1:10 Disp:oil	15.3	29.0	43.8	171.8	1328.9	1220.9	1199.9
1:10 Disp:oil	10.5	21.8	35.4	162.4	1425.7	946.4	917.3
Mean value	12.0	26.6	43.1	173.0	1351.4	1015.6	991.8
Std Error	1.7	2.4	4.3	6.6	38.1	104.5	105.5
Oil only	10.6	20.4	41.0	141.1	1458.7	1117.0	1098.5
Oil only	14.0	28.8	51.0	148.6	2045.4	1388.6	1164.7
Oil only	10.1	16.8	31.3	169.7	1601.1	970.5	894.4
Mean value	11.6	22.0	41.1	153.1	1701.7	1158.7	1052.5
Std Error	1.2	3.5	5.7	8.6	176.7	122.5	81.3
Disp only	9.2	43.4	110.0	143.0	1311.5	911.1	581.7
Disp only	9.0	45.8	131.5	182.0	1625.4	1156.9	1057.6
Disp only	9.5	49.6	125.3	161.3	992.7	941.7	823.7
Mean value	9.2	46.3	122.2	162.1	1309.9	1003.2	821.0
Std Error	0.1	1.8	6.4	11.3	182.6	77.3	137.4
nothing added	10.7	13.7	39.3	188.3	1583.4	1051.6	927.1
nothing added	8.3	12.8	33.0	167.9	1224.6	829.9	771.0
nothing added	9.0	13.7	38.3	170.7	1558.7	1063.3	928.3
Mean value	9.3	13.4	36.9	175.7	1455.6	981.6	875.5
Std Error	0.7	0.3	2.0	6.4	115.7	75.9	52.2

µg C mineralized, cumulative

Treatment	*70.5 h*	*140.5 h*	*189 h*	*213 h*	*308 h*	*334.5 h*	*380 h*
1:20 Disp:oil	11.6	35.7	77.4	230.2	1558.8	2812.8	3915.7
1:20 Disp:oil	11.0	35.1	79.6	264.3	1329.3	2337.2	3268.0
1:20 Disp:oil	10.1	37.2	82.4	264.4	1931.4	2809.4	3690.0
Mean value	10.9	36.0	79.8	253.0	1606.5	2653.1	3624.6
Std Error	0.4	0.6	1.4	11.4	175.4	158.0	189.8
1:10 Disp:oil	10.2	39.1	89.3	274.3	1573.8	2453.3	3311.5
1:10 Disp:oil	15.3	44.3	88.1	259.9	1588.8	2809.6	4009.6
1:10 Disp:oil	10.5	32.3	67.7	230.0	1655.8	2602.2	3519.5
Mean value	12.0	38.6	81.7	254.7	1606.1	2621.7	3613.5
Std Error	1.7	3.5	7.0	13.0	25.2	103.3	206.9
Oil only	10.6	31.1	72.1	213.2	1671.8	2788.9	3887.4
Oil only	14.0	42.8	93.8	242.4	2287.8	3676.4	4841.1
Oil only	10.1	27.0	58.3	228.0	1829.0	2799.5	3693.9
Mean value	11.6	33.6	74.7	227.8	1929.5	3088.3	4140.8
Std Error	1.2	4.7	10.3	8.4	184.8	294.1	354.6
Disp only	9.2	52.6	162.6	305.6	1617.2	2528.3	3110.0
Disp only	9.0	54.8	186.3	368.3	1993.7	3150.6	4208.2
Disp only	9.5	59.1	184.4	345.6	1338.3	2280.1	3103.8
Mean value	9.2	55.5	177.7	339.9	1649.7	2653.0	3474.0
Std Error	0.1	1.9	7.6	18.3	189.9	258.9	367.1
nothing added	10.7	24.5	63.8	252.1	1835.5	2887.2	3814.2
nothing added	8.3	21.1	54.1	222.0	1446.6	2276.5	3047.6
nothing added	9.0	22.7	61.0	231.7	1790.5	2853.7	3782.0
Mean value	9.3	22.8	59.6	235.3	1690.9	2672.5	3547.9
Std Error	0.7	1.0	2.9	8.9	122.8	198.2	250.4

RADIORESPIROMETRY DATA, FRESH ANS CRUDE OIL, LOW OILING LEVEL

	µg C mineralized/day					µg C mineralized/day			
Treatment	***Dodecane***	***Hex***	***2MeNap***	***Phenanthrene***		***Dodecane***	***Hex***	***2MeNap***	***Phenanthrene***
No Sed/No Disp	6.3	0.3	8.1	2.1	***mean***	5.51	0.23	9.34	1.55
No Sed/No Disp	1.4	0.3	9.7	1.1	***std error***	1.04	0.02	0.79	0.21
No Sed/No Disp	6.1	0.2	11.2	1.4					
No Sed/No Disp	6.7	0.2	7.0	1.1					
No Sed/No Disp	7.1	0.2	10.7	2.0					
With Sed/No Disp	7.0	0.1	6.2	1.2	***mean***	5.92	0.19	7.02	0.74
With Sed/No Disp	4.6	0.1	5.4	0.7	***std error***	0.63	0.03	0.85	0.15
With Sed/No Disp	5.3	0.2	10.3	0.5					
With Sed/No Disp	7.8	0.2	6.7	0.4					
With Sed/No Disp	4.9	0.3	6.4	0.9					
No Sed/With Disp	4.7	0.1	7.4	0.1	***mean***	4.56	0.10	10.03	0.15
No Sed/With Disp	3.9	0.1	10.8	0.0	***std error***	0.31	0.01	0.99	0.05
No Sed/With Disp	4.1	0.1	12.9	0.1					
No Sed/With Disp	5.7	0.1	8.3	0.3					
No Sed/With Disp	4.5	0.1	10.7	0.3					
With Sed/With Disp	4.2	0.5	7.6	0.2	***mean***	4.37	0.18	9.38	0.27
With Sed/With Disp	3.7	0.1	9.9	0.2	***std error***	0.37	0.08	0.47	0.07
With Sed/With Disp	3.4	0.1	10.2	0.1					
With Sed/With Disp	5.1	0.1	9.4	0.3					
With Sed/With Disp	5.4	0.1	9.9	0.5					

RADIORESPIROMETRY DATA, FRESH ANS CRUDE OIL, HIGH OILING LEVEL

	μg C mineralized/day				μg C mineralized/day		
Treatment	***Hex***	***MeNap***	***Phen***		***Hex***	***MeNap***	***Phen***
NoSedNo Disp	0.2	8.4	0.9	***mean***	0.20	6.87	0.77
NoSedNo Disp	0.4	10.8	0.6	***std error***	0.05	1.87	0.11
NoSedNo Disp	0.1	9.1	1.0				
NoSedNo Disp	0.2	6.0	0.9				
NoSedNo Disp	0.2	0.1	0.4				
WithSedNoDisp	0.2	6.8	1.0	***mean***	0.18	6.79	0.71
WithSedNoDisp	0.2	10.1	0.8	***std error***	0.02	1.00	0.09
WithSedNoDisp	0.2	6.4	0.7				
WithSedNoDisp	0.1	6.8	0.6				
WithSedNoDisp	0.1	3.8	0.4				
NoSedWithDisp	0.1	7.9	0.2	***mean***	0.08	8.78	0.12
NoSedWithDisp	0.1	8.9	0.0	***std error***	0.00	0.95	0.04
NoSedWithDisp	0.1	11.0	0.2				
NoSedWithDisp	0.1	10.5	0.1				
NoSedWithDisp	0.1	5.7	0.0				
WithSedWithDisp	0.2	8.5	0.5	***mean***	0.14	9.10	0.48
WithSedWithDisp	0.2	11.6	0.5	***std error***	0.02	0.87	0.04
WithSedWithDisp	0.1	9.7	0.6				
WithSedWithDisp	0.1	9.5	0.3				
WithSedWithDisp	0.1	6.2	0.5				

RADIORESPIROMETRY DATA, WEATHERED ANS CRUDE OIL, LOW OILING LEVEL

	µg C mineralized/day				µg C mineralized/day		
Treatment	***Dodecane***	***Phen***	***Hex***		***Dodecane***	***Phen***	***Hex***
Dispersant only	4.0	0.3	0.1	***mean***	4.79	0.17	0.12
Dispersant only	4.7	0.2	0.1	***std error***	0.32	0.04	0.02
Dispersant only	4.2	0.1	0.1				
Dispersant only	5.6	0.2	0.2				
Dispersant only	5.5	0.1	0.1				
Oil only	5.9	4.1	0.4	***mean***	6.79	4.21	0.36
Oil only	6.3	6.3	0.4	***std error***	1.34	0.70	0.05
Oil only	3.0	2.9	0.2				
Oil only	11.3	5.2	0.5				
Oil only	7.4	2.5	0.3				
DISP:OIL::1:10	4.6	2.4	0.1	***mean***	5.13	0.84	0.11
DISP:OIL::1:10	6.4	0.7	0.1	***std error***	1.01	0.41	0.01
DISP:OIL::1:10	1.5	0.2	0.1				
DISP:OIL::1:10	7.4	0.5	0.1				
DISP:OIL::1:10	5.8	0.3	0.1				
DISP:OIL::1:20	6.5	0.0	0.1	***mean***	6.05	0.79	0.15
DISP:OIL::1:20	5.1	0.8	0.2	***std error***	0.48	0.30	0.02
DISP:OIL::1:20	5.4	1.3	0.1				
DISP:OIL::1:20	7.2	1.6	0.2				
DISP:OIL::1:20		0.2	0.1				

RADIORESPIROMETRY DATA, WASHED CELLS, NUTRIENT-AMENDED AND NON-AMENDED

NUTRIENT-AMENDED AND NON-AMENDED WASHED CELLS

	µg C mineralized/day			µg C mineralized/day	
Treatment	***Dodecane***	***Phen***		***Dodecane***	***Phen***
DispOnlyBH	1.7	0.2	***mean***	2.51	0.20
DispOnlyBH	3.2	0.1	***std error***	0.34	0.05
DispOnlyBH	1.7	0.4			
DispOnlyBH	3.1	0.2			
DispOnlyBH	2.9	0.1			
OilOnlyBH	4.2	3.2	***mean***	3.74	3.45
OilOnlyBH	2.0	4.0	***std error***	0.49	0.28
OilOnlyBH	4.9	2.9			
OilOnlyBH	4.2	2.9			
OilOnlyBH	3.5	4.2			
dispoil10BH	3.8	1.0	***mean***	2.88	0.87
dispoil10BH	2.6	1.0	***std error***	0.37	0.08
dispoil10BH	2.8	0.7			
dispoil10BH	3.5	0.7			
dispoil10BH	1.7	1.0			
dispoil20BH	3.0	3.7	***mean***	4.00	3.43
dispoil20BH	4.6	4.2	***std error***	0.33	0.36
dispoil20BH	3.6	3.8			
dispoil20BH	4.8	3.3			
dispoil20BH	4.1	2.1			
OilOnlySW	2.0	4.6	***mean***	4.52	4.03
OilOnlySW	6.7	4.1	***std error***	0.78	0.25
OilOnlySW	4.9	4.1			
OilOnlySW	4.0	4.2			
OilOnlySW	5.0	3.1			
dispoil10SW	3.7	0.7	***mean***	3.65	0.61
dispoil10SW	2.9	0.8	***std error***	0.25	0.06
dispoil10SW	3.8	0.4			
dispoil10SW	3.3	0.6			
dispoil10SW	4.4	0.5			