Anaerobic Biodegradation of Long-Chain *n*-Alkanes under Sulfate-Reducing Conditions

MATTHEW E. CALDWELL,[†]
ROBERT M. GARRETT,[‡]
ROGER C. PRINCE,[‡] AND
JOSEPH M. SUFLITA*,[†]

Department of Botany and Microbiology, University of Oklahoma, 770 Van Vleet Oval, Norman, Oklahoma 73019, and Exxon Research and Engineering Company, Annandale, New Jersey 08801

The ability of anaerobic microorganisms to degrade a wide variety of crude oil components was investigated using chronically hydrocarbon-contaminated marine sediments as the source of inoculum. When sulfate reduction was the predominant electron-accepting process, gas chromatographic analysis revealed almost complete *n*-alkane removal ($C_{15}-C_{34}$) from a weathered oil within 201 d of incubation. No alteration of the oil was detected in sterile control incubations or when nitrate served as an alternate electron acceptor. The amount of sulfate reduced in the oil-amended nonsterile incubations was more than enough to account for the complete mineralization of the *n*-alkane fraction of the oil; no loss of this anion was observed in sterile control incubations. The mineralization of the alkanes was confirmed using ^{14}C-14,15-octacosane ($C_{28}H_{58}$), with 97% of the radioactivity recovered as $^{14}CO_2$. These findings extend the range of hydrocarbons known to be amenable to anaerobic biodegradation. Moreover, the rapid and extensive alteration in the *n*-alkanes can no longer be considered a defining characteristic of aerobic oil biodegradation processes alone.

Introduction

Fossil fuels are the foundation of many world economies, and reliance on this energy source is unlikely to wane in future decades. Such dependency is associated with a myriad of ecological consequences; most noticeably the contamination of marine environments with crude oil or distillate fractions. Of the many responses to oil pollution, the microbial destruction of hydrocarbons is increasingly relied on to convert the contaminants to innocuous end products. In this regard, the patterns of hydrocarbon decay and overall metabolic versatility of aerobic microorganisms have been well documented (as reviewed in refs *1*−*4*). The selective utilization of alkanes over other hydrocarbon fractions is frequently observed. The preferential degradation of some hydrocarbons relative to more recalcitrant molecules represents the basis for the use of biomarkers to assess the degree of aerobic biodegradation activities (*5*−*7*). However, comparable measures for the anaerobic destruction of hydrocarbons do not exist. In fact, relatively little is known about the prospects for microbial metabolism of petroleum hydrocarbons in the absence of oxygen.

Reports on the latter topic have regularly appeared since the 1940s (as reviewed in ref *8*), although lack of stringent anaerobic technique, appropriate controls, or nonreproducibility tended to undermine the conclusions of early studies. More recently, numerous studies on the anaerobic metabolism of monoaromatic hydrocarbons linked to the consumption of a variety of potential electron acceptors have appeared (as reviewed in refs *9* and *10*). However, the fate of hydrocarbons in marine ecosystems deserves particular attention since estimates suggest that 1.7−8.8 million ton of petroleum hydrocarbon enter marine environments and estuaries each year (*11*). As these environments experience the influx of labile organic matter, microbial respiratory activity frequently depletes available oxygen reserves, particularly in sediments. As marine and estuarine waters are rich in sulfate, this mobile anion is rarely limiting and most often available as a potential terminal electron acceptor. It is quite likely, therefore, that the microbial destruction of contaminating hydrocarbons might be coupled to the reduction of sulfate in these anaerobic environments.

In this regard, the ability of sulfate-reducing bacteria to degrade a variety of pollutants is beginning to be appreciated (*12*). The degradation of mono- and polyaromatic hydrocarbons under sulfate-reducing conditions has recently been demonstrated (*9*, *10*, *13*−*18*). In addition, several pure cultures of hydrocarbonoclastic sulfate-reducing bacteria are known to metabolize *n*-alkanes. *Desulfobacterium oleovorans* (strain Hxd3) is a marine isolate that can metabolize $C_{12}-C_{20}$ *n*-alkanes (*19*). Strain TD3 can grow with individual *n*-alkanes from C_6-C_{16} coupled to sulfate reduction but could only selectively remove certain *n*-alkanes when challenged to grow on crude oil (*14*). A third marine isolate can couple $C_{14}-C_{17}$ alkane metabolism to the reduction of sulfate and hydrogen sulfide production (*20*). The anaerobic microbial destruction of *n*-alkanes (from C_{15} to C_{23}) was also noted in contaminated marine sediments amended with either diesel or jet fuel (*17*). Production of $^{14}CO_2$ from 1-^{14}C-hexadecane was inhibited by molybdate, thus implicating sulfate reduction as the predominant electron-accepting process.

We wanted to further explore the limits of anaerobic hydrocarbon metabolism and help lay the foundation for future biomarker studies to more easily evaluate the ecological significance of such transformations. We used a marine sediment known to harbor a metabolically diverse microflora capable of hydrocarbon metabolism as an inoculum (*15*, *17*, *21*) and a highly characterized but weathered crude oil (*7*, *22*) as a carbon source. The latter more closely resembles hydrocarbons that typically get deposited on beaches and in sediments following the abiotic removal of the more volatile and water-soluble components (*23*). Thus, the oil was essentially devoid of lower molecular weight *n*-alkanes and monoaromatic hydrocarbons. Herein, we report the biodegradation of $C_{15}-C_{34}$ *n*-alkanes under sulfate-reducing conditions; thus extending the upper range associated with the anaerobic biodegradation of these hydrocarbons. In addition, we note that the extent of this metabolism is comparable to what has previously only been observed in studies of aerobic hydrocarbon decay.

* To whom correspondence should be addressed; telephone: 405-325-5761; fax: 405-325-7541; e-mail: jsuflita@ou.edu.
[†] University of Oklahoma.
[‡] Exxon Research and Engineering Co.

S0013-936X(98)00108-4 CCC: $15.00
Published on Web 06/04/1998

Materials and Methods

Marine sediment from San Diego Bay, CA, was slurried (1:1 g/vol) with an artificial seawater as previously described (*21*), with the following modifications: 0.001% resazurin, 1 mM sodium sulfide, and 3 mM bicarbonate. The latter were added to the slurries from sterile anoxic stock solutions. Approximately 60 mL of slurry was placed in sterile 120-mL serum bottles and sealed with a composite rubber stopper as previously described (*24*). Slurry construction and amendments were done in an anaerobic glovebag containing a headspace of 90% N_2:10% H_2. However, the slurries were placed under a 80% N_2:20% CO_2 headspace immediately after they were removed from the glovebag. Sodium sulfate or nitrate was added to the slurries from sterile anoxic stock solutions to an initial concentration of 20 mM. Autoclaved slurries (121 °C; 30 min; three consecutive days) served as sterile controls, and benzoate (1 mM) was used as a positive control. Experiments were stored at room temperature in the dark and conducted in triplicate.

The oil was a previously characterized Alaskan North Slope crude oil that had been distilled under reduced pressure until it had lost 30% of its weight (*7*, *22*). This is the maximum weight loss seen with extensive abiotic weathering (*25*). The oil was added by syringe (600 μL) to the serum bottles under anaerobic conditions using the Hungate technique (*26*) to maintain anaerobic conditions.

Sulfate and nitrate were periodically monitored by ion chromatography using a Dionex DX500 system, an AS4A column, and an ion-suppressed CD20 conductivity detector with an eluent of 1.7 mM sodium bicarbonate−1.8 mM sodium carbonate at a flow rate of 2.0 mL/min (Dionex Corp., Sunnyvale, CA). Analysis of the oil by gas chromatography/mass spectroscopy (GC/MS) followed published procedures (*27*). Separation was performed on a Hewlett-Packard HP 5890 gas chromatograph fitted with a 30 m × 0.25 mm fused silica capillary column with 5% cross-linked phenyl methyl silicone as the stationary phase. Helium was used as the carrier gas at a flow rate of 1 mL/min. Samples of 1 μL were injected automatically by a HP 6890 injector. The column temperature was set to 45 °C for the first 4 min, increased 8 °C/min to a temperature of 270 °C, then increased 5 °C/min to 310 °C, and maintained at 310 °C for 5 min. Mass spectral data were obtained with a Hewlett-Packard 5972 mass selective detector at an electron energy of 70 eV over a mass range of 35−500 amu in the total ion mode. Spectral tuning with perfluorotributylamine followed U.S. EPA method 8270C.

To confirm anaerobic alkane metabolism and to probe the fate of the substrates, a radiolabeled C_{28} *n*-alkane (^{14}C-14,15-octacosane, ≥98%; specific activity, 21.8 mCi/mmol; Sigma Chemical Co, St. Louis, MO) was added to sterile and nonsterile sediment slurries using the artificially weathered crude oil as a carrier (0.1 mL). Slurries were incubated for an additional 90 d at room temperature in the dark and analyzed for the production of radiolabeled carbon dioxide and methane, as previously described (*28*). Residual oil was extracted from the incubations with methylene chloride, and the resulting aqueous and solvent fractions were assayed by liquid scintillation counting (*24*).

Results and Discussion

Sulfate reduction in the sediment slurries was monitored in both the presence and the absence of the oil (Figure 1). Sterile controls showed no significant loss of sulfate over the course of the incubation, while little sulfate reduction (~3 mM, 0.18 mmol) was noted in oil-unamended controls. Nonsterile incubations amended with oil or oil plus benzoate exhausted the available sulfate reserves (~25 mM, 1.5 mmol) within 125 d. The addition of benzoate as a positive control did not significantly influence the rate of sulfate reduction in the slurries (Figure 1), and HPLC analysis confirmed that this compound was depleted within 30 d (data not shown). A subsequent amendment of sulfate on day 125 (20 or 10 mM for slurries receiving oil or oil plus benzoate, respectively) was also reduced, but the rate of sulfate removal decreased after 160 d (Figure 1).

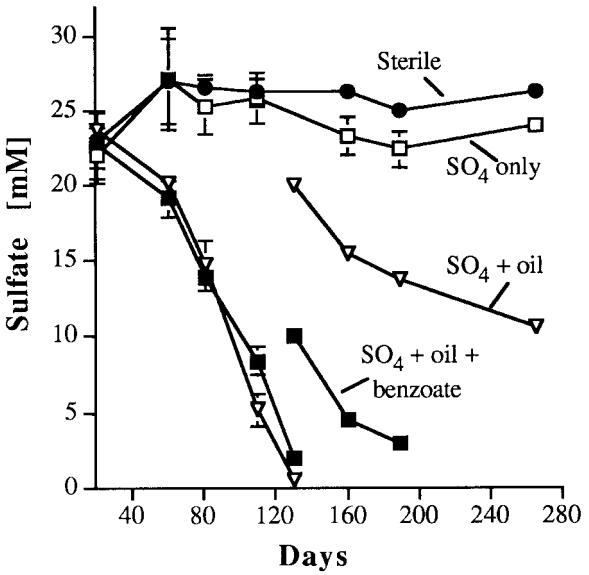

FIGURE 1. Sulfate loss in marine sediment incubations. On day 125, reamendments of 20 and 10 mM sulfate were made to incubations containing oil alone (▽) or oil plus the positive control substrate benzoate (■), respectively. Standard deviations are indicated.

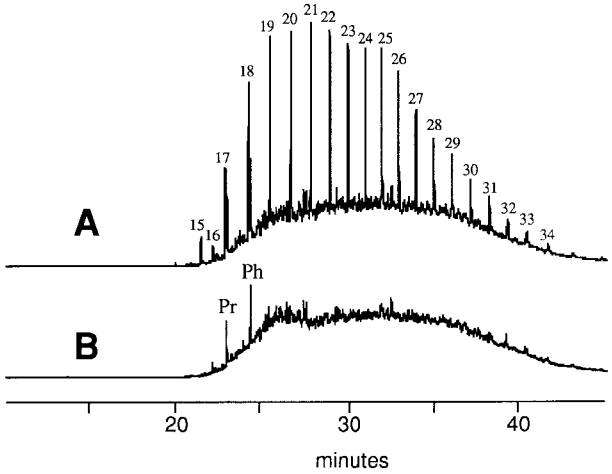

FIGURE 2. Gas chromatographic profiles of the saturated hydrocarbon fraction of weathered Alaska North Slope crude oil extracted from sterile (A) and nonsterile (B) marine sediments following a 201-d incubation. Numbers on the peaks represent chain lengths of *n*-alkanes; Pr, pristane; Ph, phytane.

For slurries amended with nitrate, 90% of the electron acceptor was depleted within the first 60 d in all nonsterile incubations, regardless of whether they were also amended with oil or with the positive control benzoate (data not shown). Nitrate depletion could not be correlated with either benzoate or oil removal. No loss of nitrate was noted in the sterile incubations. However, the loss of nitrate could be correlated with an increase in sulfate. That is, sulfate accumulated from ~6 to 11−15 mM in nonsterile incubations, whereas no such increase was detected in sterile controls. The microbial oxidation of reduced sulfur species coupled with the reduction of nitrate is known (*29*), and the

TABLE 1. Recovery of $^{14}C^a$-Labeled Components Incubated with ^{14}C-14,15-Octacosane from San Diego Bay Sediments after 90 d

incubation	starting amendment	$^{14}CO_2$ recovered	solvent recovery	aqueous recovery	total recovery
sterile	5.5×10^6	BDL[b]	5.2×10^6	1.6×10^4	5.2×10^6 (95%)
live	5.5×10^6	5.6×10^6	2.4×10^5	9.0×10^3	5.8×10^6 (105%)

[a] All values reported in dpm. [b] BDL, below detection limit.

incubations were visibly less colored, presumably due to this process.

The physical appearance of the sulfate- and oil-amended slurries changed as the incubation proceeded. Even though the oil was applied as an immiscible layer, small pockets of sediment blackening were easily observed in nonsterile incubations. Apparently, the sediment blackening was due to the production of iron sulfide minerals. However, it is unclear whether the spatially discreet sulfate reduction zones were due to the patchy distribution of the requisite bacteria or if droplets of oil were trapped within the sediment matrix and stimulated sulfate reduction activity where deposited. During incubation it became increasingly more difficult to discern the oil-immiscible layer, due to both the apparent breakup of the oil layer and the overall blackening of sediment slurries. Comparable physical changes were not evident in either the sterile or oil-unamended controls.

After 201 d of incubation, slurries were extracted and analyzed by gas chromatography to characterize residual oil constituents. No qualitative changes in oil composition could be detected in sterile control incubations (Figure 2A) relative to the starting oil (not shown). Additionally, no changes in oil composition were observed in nonsterile nitrate-amended incubations over this same time period (data not shown). In contrast, the *n*-alkanes were almost completely removed in nonsterile oil-amended incubations (with or without benzoate) when sulfate served as the terminal electron acceptor (Figure 2B). The apparent loss of these hydrocarbons coupled with the depletion of sulfate is consistent with the anaerobic biodegradation of these constituents.

To test this hypothesis, radiolabeled octacosane (^{14}C-14,-15-$C_{28}H_{58}$) was added to the slurries and assays for radiolabeled hydrocarbon and mineralized end products (CO_2 and CH_4) were conducted after an additional 90 d incubation period. No transformation of octacosane to radiolabeled CO_2 or CH_4 could be detected in the sterile controls (Table 1). In fact, 95% of the applied radiolabel was recovered in the solvent used to extract residual hydrocarbon components. In contrast, approximately 97% of the radioactivity could be recovered as $^{14}CO_2$ in nonsterile incubations with only traces in the solvent extract (Table 1). No recovery of $^{14}CH_4$ was obtained from any incubation.

The gas chromatographic profiles (Figure 2) clearly indicate that a diverse range of *n*-alkanes, up to 34 carbons in chain length, could be biologically removed from a weathered crude oil upon incubation with sediment-associated marine anaerobes under sulfate-reducing conditions. These transformations did not occur when nitrate was provided as a potential terminal electron acceptor. Although nitrate has shown promise as a potential tool in the anaerobic bioremediation of monoaromatic hydrocarbons (*9*, *10*, *30*, *31*), the effectiveness of nitrate is almost exclusively observed in freshwater or aquifer material. Additionally, the lack of monoaromatics in our weathered oil would preclude their biodegradation in our incubations. Zeyer and colleagues (*32*, *33*) have indicated that nitrate-reducing microorganisms associated with sediment from a freshwater aquifer were able to effectively degrade 50% of a weathered diesel oil and the isoprenoid molecule, pristane. Though these reports open the possibility for nitrate-coupled biodegradation of heavier oil components, no loss of pristane or other compounds was indicated in extracts of our live incubations. This is not entirely surprising since marine sediments are considered low nitrate environments.

The amount of sulfate reduced in our incubations helps suggest the ultimate fate of these hydrocarbons. At the time the residual oil was extracted, approximately 30 mM sulfate (1.8 mmol) was removed in nonsterile incubations relative to the oil-unamended controls (Figure 1). The total amount of *n*-alkanes in this crude oil is known (*22*), as was the quantity of oil added to the incubation mixtures. On the basis of the theoretically expected stoichiometry (comparable to the estimates made in ref *19*), we calculate that only 24 mM sulfate (1.44 mmol) would be required for the complete conversion of all the *n*-alkanes to carbon dioxide. Thus, more than enough sulfate was reduced to account for the overall mineralization of the alkanes. The amount of electron acceptor depleted in excess of that theoretically expected probably indicates that other hydrocarbon components in the oil can also be anaerobically metabolized.

Our suggestion of alkane mineralization linked to sulfate reduction is consistent with the findings of Widdel and colleagues (*14*, *19*). They measured close to theoretically expected amounts of sulfide produced in cultures of *Desulfobacterium oleovorans* (strain Hxd3) metabolizing hexadecane and strain TD3 growing on decane. Similarly, So and Young (*20*) also obtained a marine bacterium capable of coupling the degradation of hexadecane to sulfate reduction. Such comparisons of the observed versus the expected amounts of sulfate required for alkane decay provide strong suggestive evidence for the mineralization of the oil associated *n*-alkanes. However, this contention was confirmed by our recovery of the vast majority (97%) of the radioactivity originally associated with ^{14}C-octacosane as $^{14}CO_2$ in nonsterile incubations, whereas no significant amount of label was found in the carbon dioxide traps for sterile controls (Table 1).

Sulfate-containing samples incubated for 201 d exhibited a 40% loss of the isoprenoid molecules pristane and phytane relative to sterile controls. The addition of benzoate reduced pristane and phytane degradation by 50%. No significant degradation of polycyclic aromatic hydrocarbons was noted at 201 d. Samples incubated for 365 d exhibited a 70% loss of pristane and phytane in incubations without benzoate, approximately a 50% loss in its presence. In addition dimethylnaphthalene and phenanthrene were degraded 25% in the 365 d samples, suggesting that while the preference of the inoculum is for straight chain saturates, the anaerobic degradation of aromatic compounds will proceed in the absence of preferred substrates. While degradation of more complex aromatic compounds (dibenzothiophene and chrysene, for example) was not observed at 365 d, it is possible that these substrates would be utilized given additional time and sufficient electron acceptor. In this regard, it should be noted that Coates and Lovely found that polynuclear hydrocarbons were amenable to anaerobic biodegradation using the same inoculum (*15*, *17*). However, when exposed to a complex crude oil, the degradation of polynuclear hydrocarbons was clearly less important than the degradation of alkanes in terms of the overall mass loss of the hydrocarbons.

Several investigators have relied on the production of $^{14}CO_2$ from radiolabeled substrates to assess mineralization of *n*-alkanes under anaerobic conditions. Davis and Yarbrough (*34*) observed small amounts of $^{14}CO_2$ from radiolabeled octadecane after 22 d of incubation with *Desulfovibrio desulfuricans*. However, biodegradation was not coupled to sulfate reduction, and the amounts of radioactivity recovered were small enough to raise questions about the radiopurity of the starting substrate. Similarly, Ward and Brock (*35*) observed 5.6% recovery of $^{14}CO_2$ from 1-^{14}C-hexadecane incubated with anoxic sediments from Lake Mendota, WI. Formaldehyde-inhibited controls exhibited a similar recovery of radioactivity, and the process was not stimulated by the addition of nitrate or sulfate. In a more recent study, Coates et al. (*17*) observed substantial recovery (>70%) of $^{14}CO_2$ from radiolabeled hexadecane in marine sediments from San Diego Bay, CA. Inhibition of this metabolism by molybdate (20 mM) implicated sulfate reduction as the major terminal electron-acceptor process in these incubations.

The use of ^{14}C-14,15-octacosane allowed us to address two important points. First, we could show that even a higher molecular weight alkane was amenable to anaerobic attack. Prior to our study, the highest molecular weight alkane known to biodegrade under anaerobic conditions was C_{23} (*17*). Second, with the radiocarbon on the interior of the molecule and its eventual recovery as $^{14}CO_2$, we confirmed that the alkane must have been substantially mineralized as opposed to its incorporation into cell biomass.

The total recovery of radiolabel in the incubations was generally very high (Table 1) with disparities explained by the difficulties in accurately dispensing 100 μL of the weathered Alaska North Slope crude oil used a carrier. For instance, in one replicate we recovered 99% of the label as $^{14}CO_2$, but showed a total recovery of 165% of the expected amount of applied radiolabel (data no shown). However, even with this replicate, almost all radioactivity was recovered as carbon dioxide and confirmed that the octacosane was mineralized under sulfate-reducing conditions.

The importance of these findings is worth noting. When assessing the biodegradation of petroleum hydrocarbons in the environment, it is generally presumed that the aerobic microbial metabolism of these substrates is the predominant removal mechanism (*1*−*4*). In fact, it is commonly observed that alkanes are preferentially utilized by aerobic microorganisms relative to other components when oily mixtures of hydrocarbons are available to the resident microflora (*7*, *22*, *36*−*38*), although exceptions have been seen (*39*, *40*). The former is the case in experiments using the same weathered oil where aerobic alkane biodegradation is essentially complete within 14 d of incubation (*7*). Our observations call such generalizations into question. That is, there apparently are environmental compartments where the anaerobic microbial metabolism of hydrocarbons may represent an equally important fate process for these materials. Moreover, if the rather dramatic preferential utilization of the alkanes observed in our experiments proves general, conclusions on the importance of aerobic biodegradation based on the patterns of hydrocarbon decay may at least be called into question.

As noted above, the utilization of *n*-alkanes under sulfate-reducing conditions has been observed before, but the diversity and extent of this metabolism documented herein has not been previously recognized. It thus appears that the capacity of anaerobes to metabolize hydrocarbons must be reevaluated when considering the transport and fate of oily materials in the environment. Moreover, a reexamination of the role of these organisms in such diverse areas as the souring of oil reservoirs (*41*, *42*), the fouling of petroliferous formations (*43*, *44*), the corrosion of oil field equipment (*45*, *46*), and their potential to remediate oily waste components would seem appropriate.

Acknowledgments

Research at the University of Oklahoma was supported by the Office of Naval Research (N00014-95-1-0956) and the American Petroleum Institute. The results and conclusions are those of the authors and not necessarily those of the funding agencies. We would like to thank J. Coates and D. Lovley for providing the marine sediments used is this study.

Literature Cited

(1) Leahy, J. G.; Colwell, R. R. *Microbiol. Rev.* **1990**, *54*, 305.
(2) Atlas, R. M.; Bartha, R. *Adv. Microb. Ecol.* **1992**, *12*, 287.
(3) Prince, R. C. *Crit. Rev. Microbiol.* **1993**, *54*, 217.
(4) Rosenberg, E.; Ron, E. Z. In *Bioremediation: Principles and Applications*; Crawford, R., Crawford, D., Eds.; Cambridge University Press: Cambridge, 1996; pp 100−124.
(5) Butler E. L.; Prince, R. C.; Douglas, G. S.; Aczel, C.; Hsu, C. S.; Bronson, M. T.; Clark, J. R.; Lindstrom, J. E.; Steinhauer, W. G. In *On-Site Bioreclamation: Processes for Xenobiotic and Hydrocarbon Treatment*; Hinchee, R. E., Olfenbuttel, R. F., Eds.; Butterworth-Heinemann: Boston, MA, 1991; pp 515−521.
(6) Douglas, G. S.; McCarthy, K. P.; Dahlen, D. T.; Seavey, J. A.; Steinhauer, W. G.; Prince, R. C.; Elmendorf, D. L. In *Contaminated Soils; Diesel Fuel Contamination*; Kostecki, P. T., Calabrese, E. J., Eds.; Lewis Publishers: Boca Raton, FL, 1992; pp 1−21.
(7) Prince, R. C.; Elmendorf, D. L.; Lute, J. R.; Hsu, C. S.; Haith, C. E.; Senius, J. D.; Dechert, G. J.; Douglas, G. S.; Butler, E. L. *Environ. Sci. Technol.* **1994**, *28*, 142.
(8) Zobell, C. E. *Bacteriol. Rev.* **1946**, *10*, 1.
(9) Krumholz, L. R.; Caldwell, M. E.; Suflita, J. M. In *Bioremediation: Principles and Applications*; Crawford, R., Crawford, D., Eds.; Cambridge University Press: Cambridge, 1996; pp 61−99.
(10) Lovley, D. R. *J. Ind. Microbiol. Biotechnol.* **1997**, *18*, 75.
(11) National Academy of Sciences. *Oil in the Sea: Inputs, Fates, and Effects*; National Academy Press: Washington, DC, 1985.
(12) Ensley, B. D.; Suflita, J. M. In *Sulfate-Reducing Bacteria*; Barton, L., Ed.; Plenum Press: New York, 1995; pp 293−332.
(13) Beller, H. R.; Spormann, A. M.; Sharma, P. K.; Cole, J. R.; Reinhard, M. *Appl. Environ. Microbiol.* **1996**, *62*, 1188.
(14) Rueter, P.; Rabus, R.; Wilkes, H.; Aeckersberg, F.; Rainey, F. A.; Jannasch, H. W.; Widdel, F. *Nature* **1994**, *372*, 455.
(15) Coates, J. D.; Anderson, R. T.; Lovley, D. R. *Appl. Environ. Microbiol.* **1996**, *62*, 1099.
(16) Bedessem, M. E.; Swoboda-Colberg, N. G.; Colberg, P. J. S. *FEMS Microbiol. Lett.* **1997**, *152*, 213.
(17) Coates, J. D.; Woodward, J.; Allen, J.; Philp, P.; Lovley, D. R. *Appl. Environ. Microbiol.* **1997**, *63*, 3589.
(18) Zhang, X.; Young, L. Y. *Appl. Environ. Microbiol.* **1997**, *63*, 4759.
(19) Aeckersberg, F.; Bak, F.; Widdel, F. *Arch. Microbiol.* **1991**, *156*, 5.
(20) So, C. M.; Young, L. Y. In *Abstracts of the 97th Annual Meeting of the American Society for Microbiology*; American Society for Microbiology: Washington, DC, 1997; p 493, Q-230.
(21) Lovley, D. R.; Coates, J. D.; Woodward, J. C.; Phillips, E. J. P. *Appl. Environ. Microbiol.* **1995**, *61*, 953.
(22) Elmendorf, D. L.; Haith, C. E.; Douglas, G. S.; Prince, R. C. In *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds*; Hinchee, R. E., Leeson, A., Semprini, L., Ong, S. K., Eds.; Lewis Publishers: Boca Raton, FL, 1994; pp 188−202.
(23) Whitman, B. T.; Duckworth, D. F.; Harding, A. A. B.; Jeffrey, P. G.; Perry, S. C. In *Marine Pollution by Oil: Characterization of pollutants, sampling, analysis and interpretation*; Applied Science: London, 1975.
(24) Kazumi, J.; Caldwell, M. E.; Suflita, J. M.; Lovley, D. R.; Young, L. Y. *Environ. Sci. Technol.* **1997**, *31*, 813.
(25) Payne, J. R.; Clayton, J. R.; McNabb, G. D.; Kirstein, B. E. In *Proceedings of the 1991 International Oil Spill Conference*; American Petroleum Institute: Washington, DC, 1991; pp 641−654.
(26) Hungate, R. E. In *Methods in Microbiology*, Vol. 3B; Norris, J. R., Ribbons, D. W., Eds.; Academic Press: New York, 1969; pp 117−132.
(27) Douglas, G. S.; McCarthy, K. J.; Dahlen, D. T.; Seavey, J. A.; Steinhauer, W. G.; Prince, R. C.; Elmendorf, G. L. *J. Soil Contam.* **1992**, *1*, 197.
(28) Nuck, B. A.; Federle, T. W. *Environ. Sci. Technol.* **1996**, *30*, 3597.

(29) Fuseler, K.; Krekeler, D.; Sydow, U.; Cypionka, H. *FEMS Micriobiol. Lett.* **1996**, *144*, 129.
(30) Alvarez, P. J. J.; Vogel, T. M. *Water Sci. Technol.* **1995**, *31*, 15.
(31) Langenhoff, A. A. M.; Zehnder, A. J. B.; Schraa, G. *Biodegradation* **1996**, *7*, 267.
(32) Bregnard, T. P.-A.; Höhener, P.; Häner, A.; Zeyer, J. *Environ. Toxicol. Chem.* **1996**, *15*, 299.
(33) Bregnard, T. P.-A.; Häner, A.; Höhener, P.; Zeyer, J. *Appl. Environ. Microbiol.* **1997**, *63*, 2077.
(34) Davis, J. B.; Yarbrough, H. F. *Chem. Geol.* **1966**, *1*, 137.
(35) Ward, D. M.; Brock, T. D. *Geomicrobiol. J.* **1978**, *1*, 1.
(36) Higgins, I. J.; Gilbert, P. D. In *The Oil Industry and Microbial Ecosystems*; Chater, K. W. A., Somerville, H. J., Eds.; Hyden Press: London, 1978; pp 80−117.
(37) McMillen, S. J.; Gray, N. R.; Kerr, J. M.; Requejo, A. G.; McDonald, T. J.; Douglas, G. S. In *Monitoring and Verification of Bioremediation*; Hinchee, R. E., Douglas, G. S., Ong, S. E., Eds.; Batelle Press: Columbus, OH, 1995; pp 1−9.
(38) Kaplan, I. R.; Galperin, Y.; Alimi, H.; Lee, R.; Lu, S. *Ground Water Monit. Rem.* **1996**, *16*, 113.
(39) Fedorak, P. M.; Westlake, D. W. S. *Can. J. Microbiol.* **1981**, *27*, 432.
(40) Fayad, N. M.; Overton, E. *Mar. Pollut. Bull.* **1995**, *30*, 239.
(41) Odom, J. M. In *The Sulfate-Reducing Bacteria: Contemporary Perspectives*; Odom, J. M., Singleton, R., Jr., Eds.; Springer-Verlag: New York, 1993; pp 189−249.
(42) Jack, T. R.; Westlake, D. W. S. In *Sulfate-Reducing Bacteria*; Barton, L., Ed.; Plenum: New York, 1995; pp 265−292.
(43) Herbert, B. N. In *Microbial Problems in the Offshore Oil Industry*; Hill, E. C., Shennan, J. L., Watkinson, R. J., Eds.; Wiley: Chichester, 1987; pp 63−73.
(44) Cusack, F.; Brown, D. R.; Costerton, J. W.; Clementz, D. M. *J. Petrol. Sci. Eng.* **1987**, *1*, 39.
(45) Dexter, S. C., Ed. *Biologically Induced Corrosion*; National Association of Corrosion Engineers: Houston, TX, 1986.
(46) Hamilton, W. A.; Whonchee, L. In *Sulfate-Reducing Bacteria*; Barton, L., Ed.; Plenum: New York, 1995; pp 243−264.

Received for review February 2, 1998. Revised manuscript received April 22, 1998. Accepted May 13, 1998.

ES9801083