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Mechanisms of Intrinsic Bioremediation of Gas Condensate Hydrocarbons in Saturated Soil

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ABSTRACT

The addition of gas condensate hydrocarbons to saturated soil from a gas production site stimulated sulfate reduction under anaerobic and oxygen-limiting conditions, and nitrate and Fe(III) reduction under oxygen-limiting conditions, compared to biotic controls that lacked hydrocarbon and sterile controls. The sulfate reduction corresponded to a reduction in the amount of toluene relative to other hydrocarbons. These results suggest that subsurface soils at the gas production site have the potential for intrinsic bioremediation of hydrocarbons.

Index Entries: Intrinsic bioremediation; gas condensate; anoxic; oxygen-limited; hydrocarbons.

INTRODUCTION

Soil and ground water contamination by petroleum hydrocarbons is an ongoing environmental problem. The benzene, toluene, ethylbenzene, and xylene (BTEX) from a hydrocarbon spill or leak are of special interest because they are relatively water soluble, and two of these components have been associated with known health risks. Benzene is a confirmed carcinogen, and toluene is a depressant of the central nervous system *(1)*. Recently, researchers have convincingly demonstrated the natural attenuation of hydrocarbon plumes in ground water under anaerobic or microaerophilic, as well as aerobic conditions. Nitrate, iron(III) oxides, and sulfate have all been identified as potential electron acceptors for hydrocarbon degradation in the absence of oxygen *(2,3)*.

Gas condensate liquids were found to contaminate the soil and ground water at some gas production sites operated by Amoco in the Denver basin. Two of these sites have been closely monitored since July 1993 to determine if intrinsic aerobic or anaerobic bioremediation of hydrocarbons occurs at a sufficient rate and to an

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adequate end point to support a no-intervention decision. The limited migration of the highly soluble BTEX components and the depletion of several potential electron acceptors in the contaminated zone vs the uncontaminated areas suggest that intrinsic bioremediation is occurring at the sites by multiple pathways, including aerobic oxidation, sulfate reduction, and possibly by Fe(III) reduction *(4)*.

Laboratory investigations were conducted to determine whether the depletion of electron acceptors in the presence of high hydrocarbon concentrations at these sites was biologically mediated and whether the presence of hydrocarbon stimulated the use of these electron acceptors. Four different electron acceptors were investigated: nitrate, sulfate, Fe(III), and carbon dioxide (methanogenic), under anoxic and limited oxygen conditions. Saturated soil microcosms with excess hydrocarbon were used to simulate the field conditions as closely as possible.

The objective of this project is to develop a computational model that can be used to predict the rate and end point of the natural attenuation of hydrocarbons at these and similar sites. The ultimate goal of this research is to be able to use this computational model to evaluate the environmental risks of a no-intervention decision when soil and ground water are contaminated with light hydrocarbons containing BTEX.

MATERIALS AND METHODS

Composition of Saturated Soil Microcosms

Microcosm studies used 25 g of native soil obtained from an uncontaminated region of the Denver basin field site. Native soil was dried for about 24 h at 70°C to facilitate handling and then sieved through a standard 10-mesh sieve to remove larger stones and root ends. The inoculum was from the contaminated region and comprised about 10% (by wt) of the native soil used in the microcosms. The contaminated soil was a mixture of cores obtained from both saturated and vadose zones from a region downstream of the gas condensate spill, including regions with free product and from the ground water plume area *(5)*. Soil for use as inoculum was collected using precautions to maintain anaerobic conditions throughout the sampling procedure. Mineral and electron acceptor analyses of native soil and the soil inoculum are given in Table 1.

The soil was saturated with 6 mL of a mineral salts medium, which was composed of 1.0 mL of trace metals solution, 2.0 mL of mineral solution, and 0.35 g of sodium bicarbonate/100.0 mL of medium. The compositions of trace metals solution and mineral stock solution are given by Tanner et al. *(6)*. Electron acceptors were added to the mineral salts medium before saturating the soil. Amended nitrate and sulfate concentrations in the nitrate- and sulfate-amended microcosms were 0.168 mmol/microcosm and 0.144 mmol/microcosm, respectively. An internal standard, sodium bromide (0.138 mmol/microcosm), was used to account for any losses of anions to the soil. Iron(III) were added as a separate phase in the form of an amorphous oxyhydroxide gel *(7)* to give 0.108 mmol of amended Fe(III)/microcosm. The medium (mineral salts + electron acceptor) was anaerobically prepared using a Hungate gassing station *(8,9)* and transferred to an anaerobic chamber. The medium was then added to serum bottles containing the native soil with or without soil inoculum within the anaerobic chamber. The bottles were stoppered with Teflon™-lined gray butyl rubber stoppers (Wheaton, Milleville, NJ) and the headspace exchanged from anaerobic chamber gas to 80% N_2 + 20% CO_2 *(9)*. These

Table 1
Mineral and Electron Acceptor Analyses
of the Solid Media, Saturated Soil Experiment

	Native soil	Soil inoculum	Ottawa sand
Minerals			
Quartz	63%	63%	100%
Feldspar	23%	23%	0
Clays	10%	10%	0
Other	Dolomite, siderite, gypsum	Dolomite, siderite, gypsum	–
Leachable electron acceptors,[a] mg/kg (dry basis)			
Nitrate	11.8	0.0	0.0
Sulfate	85.2	49.2	0.0
Fe(III)	839.1	1024.4	39.5
Fe(II)	356.5	2362.2	20.8

[a]Leachable electron acceptors: The solid media were extracted with an equal amount (by wt) of 1*N* HCl over a period of 48 h at room temperature without any shaking, and the extract filtered and analyzed to obtain the leachable electron acceptors.

will be referred to as the anoxic microcosms. A second identical set of microcosms was prepared outside the anaerobic chamber, and the headspace exchanged to 21% O_2, 20% CO_2 with the balance being N_2. The total millimoles of oxygen in the gas phase was 0.258 mmol/microcosm, with 0.0016 mmol in the aqueous phase. Carbon dioxide was included in the headspace of all microcosms as a component of the buffering system (with bicarbonate in the aqueous phase) and as a potential electron acceptor in microcosms that did not receive exogenous electron acceptors. The pH of the aqueous phase under these conditions was 7.2.

Since the native soil and soil inoculum used in these experiments contained significant amounts of the potential electron acceptors, sulfate and Fe(III) (Table 1), an additional series of experiments were done using 25 g of clean Ottawa sand in place of native soil. The background levels of electron acceptors in the Ottawa sand were much lower (Table 1).

Three types of microcosms were prepared: biotic, sterile, and background. The biotic microcosms contained 25 g of native soil or Ottawa sand with 10% (by wt) soil inoculum saturated with the mineral salts medium with or without exogenous electron acceptor and with sufficient condensate to give a hydrocarbon concentration of 10,000 mg/kg of soil or sand on a dry-wt basis. The sterile controls were prepared in an identical manner, but were steam sterilized at 121°C for 60 min. The background controls consisted of native soil or Ottawa sand with the mineral salts medium with and without exogenous electron acceptor and did not contain any hydrocarbon or soil inoculum.

The gas condensate obtained from the Denver basin site was heated to remove part of the low boiling compounds to reduce the potential for volatile losses during the experiment. The gas condensate was heated using a Soxtec Heating System 2 HT2 (Perstorp Analytical Inc., Silver Spring, MD) for a period of 8 h at temperatures up to 110°C. The heat-treated condensate was filter-sterilized using a 0.22-μ, sterile,

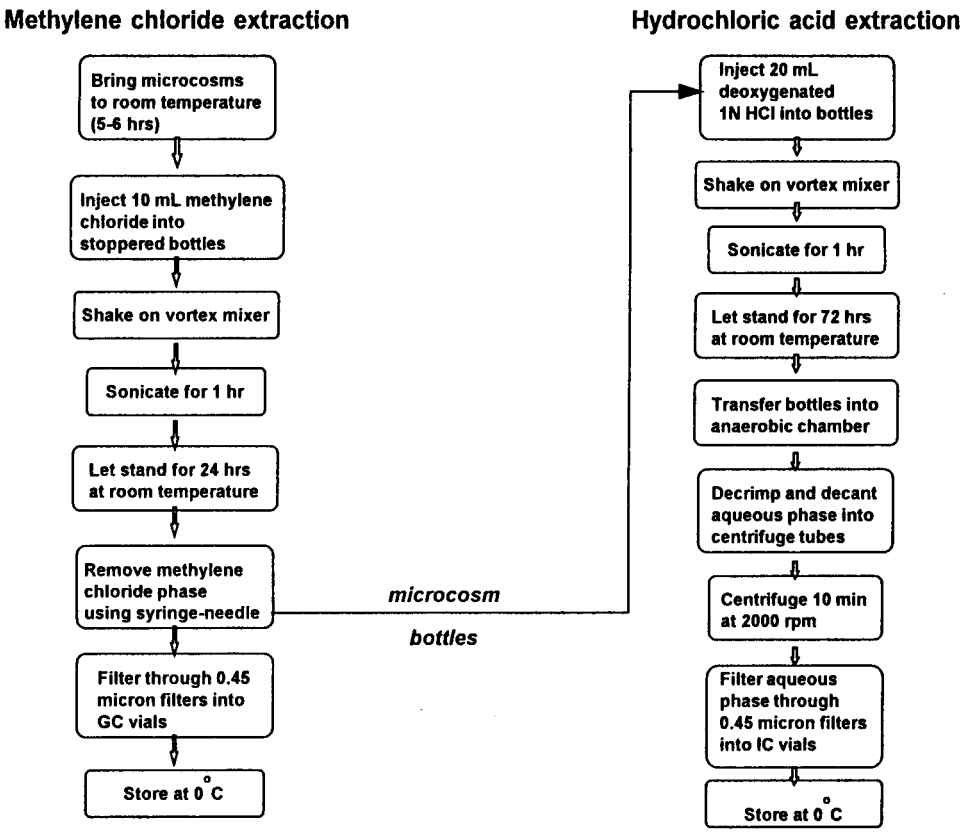

Fig. 1. Experimental protocol used for the analysis of hydrocarbons and electron acceptors in microcosms.

cartridge filter (Gelman Sciences, Ann Arbor, MI), and then injected into each microcosm using a sterile syringe and needle. The microcosms were then shaken manually to mix the hydrocarbon with the soil and the aqueous phase, and transferred into anaerobic jars with a gas phase of 80% N_2 + 20% CO_2.

Ground water temperatures at the Denver basin field site range from 6–20°C owing to seasonal variations. The microcosm experiments reported here were carried out at an incubation temperature of 10°C. Triplicate microcosms of each type were sacrificed at six different times over a period of 402 d. The contents were analyzed for electron acceptors, hydrocarbons, and possible products of hydrocarbon degradation.

Analytical

Microcosms were extracted with methylene chloride for hydrocarbon analysis, and then with 1*N* HCl *(7)* to produce an aqueous phase for analysis of electron acceptors and possible products of biodegradation (low molecular weight organic acids). The extraction and sampling protocol is given in Fig. 1. Hexacosane was used as an internal standard (300 mg/ kg) during the methylene chloride extraction to account for any losses during the extraction procedure.

Total petroleum hydrocarbons (TPH) was obtained by gas chromatography; each chromatogram resulting from analysis of the methylene chloride extract was

integrated using baseline root integration and calibrated using an external standard made with heat-treated condensate to obtain the TPH (C6-C20). Data were normalized to account for recovery of the hexacosane internal standard.

Methylene chloride extract was analyzed for hydrocarbons on an HP 5880 gas chromatograph equipped with a flame ionization detector. A DB-1 capillary column was used with an injection temperature of 290°C and detector temperature of 345°C. The column was subjected to the following temperature program: prehold at 35°C for 1 min, gradient of 7°C/min from 35 to 340°C, posthold at 340°C for 10 min. Helium was used as a carrier gas with split injection at a split ratio of 1:100.

Nitrate, sulfate, bromide, and acetate were analyzed on a Dionex ion chromatograph (IC) model AGP-1 equipped with electrochemical conductivity detector. A 4-mm IonPak AS5 Dionex analytical column was used to carry out the separation. A gradient method was used with sodium hydroxide solution containing 5% methanol as the eluant. The sodium hydroxide concentration was ramped from 1 to 64 m*M* over a period of 22 min, at the end of which the eluant flowed isochratically at a concentration of 64 m*M* for a period of 3 min, followed by a gradient with sodium hydroxide concentration dropping from 64 to 1 m*M* over a period of 3 min. The total run time was 45 min with isochratic flow of 1 m*M* NaOH for the last 17 min. The eluant flow rate was 1 mL/min. A Dionex membrane suppresser was used with sulfuric acid as the reagent at a concentration of 25 m*M* and flow rate of 10 mL/min. Data were normalized to account for recovery of the bromide internal standard.

Fe(II) and Fe(III) were separated on a Dionex IonPak CS5 analytical column using a second IC instrument. The eluant was 50 m*M* acetic acid-50 m*M* sodium acetate solution with 10% methanol containing a chelating agent, 2,6-pyridine dicarboxylic acid (PDCA) at a concentration of 6 m*M*. The eluant flow rate was 1 mL/min. The metals were detected by measuring the absorbance of a complex formed with a postcolumn reagent, 1*M* acetic acid 3-*M* ammonium hydroxide containing 0 3 m*M* 4-(2-pyridylazo) resorcinol, monosodium salt hydrate (PAR) flowing at 0.7 mL/min.

The low-mol-wt organic acids were separated using a Dionex IonPak NS1 analytical column on a third IC instrument and quantified using a UV detector. A gradient method was used with 100% solution A going to 100% solution B over a period of 35 min. Solution A was 0.025 m*M* HCl in 24% CH_3CN–6% MeOH solution, and solution B was 0.05 m*M* HCl in 60% CH_3CN–24% MeOH solution. The eluant followed a gradient to 100% A over the next 3 min. The eluant flow rate was 1.5 mL/min. A Dionex membrane suppresser was used with 2.5 m*M* KOH as the reagent at a flow rate of 2 mL/min. Separation of propionic acid to decanoic acid was achieved with this method in 35 min.

RESULTS AND DISCUSSION

Apparent Loss of Hydrocarbon

Because of an excess of hydrocarbon relative to the amount of electron acceptor present (total amount of electron acceptor present in the microcosms was sufficient for complete oxidation of only about 1–2% of the hydrocarbon added to each microcosm), the hydrocarbon loss could not be used as a measure of biological activity. A 60–70% loss in TPH occurred in all microcosms during the first 40 d of incubation, including the sterile controls, under all electron acceptor conditions The TPH concentrations remained relatively unchanged thereafter. This large total loss in hydro-

carbon is not a result of biotic processes, since similar losses were observed in sterile and nonsterile microcosms. A comparison of gas chromatograms from d 0 and 402 (data not shown) showed the presence of the light hydrocarbons in the d 402 chromatogram with relatively small losses compared to the high boiling compounds. This eliminates volatilization as a possible mechanism for TPH loss of the magnitude observed.

Adsorption of the hydrocarbon on the solid matrix is another possible explanation for the apparent disappearance of hydrocarbon. The methylene chloride extraction procedure was unchanged over the period of the experiment; however, the extraction efficiency may have been reduced owing to penetration of the hydrocarbon into the micropores of the soil/sand. To test this, the soil from a biotic microcosm that had been extracted with methylene chloride and acid was further extracted with methylene chloride using a Soxtec method for 8 h. The amount of hydrocarbon recovered from the second extraction was only about 8.3% of the amount of TPH lost.

Anoxic Conditions

About 600–800 mg/(kg soil) (dry wt) Fe(III) were present in the sulfate-amended, anoxic microcosms. Fe(III) reduction with Fe(II) production occurred simultaneously with sulfate reduction. At the end of 402 d, approx 500 mg/kg Fe(III) were consumed and 800 mg/kg of Fe(II) were produced. The background soil microcosms without hydrocarbon were similar to the biotic microcosms with hydrocarbon, indicating that Fe(III) reduction was linked to oxidation of a background carbon source. Native soil contained about 1000 mg and the soil inoculum contained about 900 mg of organic carbon/kg (dry wt) of soil (Leco Furnace test). No Fe(III) reduction was observed in any anoxic, sand microcosms.

In the sulfate-amended, anoxic soil microcosms, a reduction in sulfate concentration occurred after 122 d and sulfate was almost completely consumed by the end of 402 d (Fig. 2). Sulfate depletion was observed only in biotic, soil microcosms with hydrocarbon added, and not in background microcosms that lacked hydrocarbon or sterile microcosms. This suggests that sulfate reduction may be linked to hydrocarbon oxidation. Moreover, about a 38% loss in the amount of toluene present was observed in the biotic, soil microcosms compared to the sterile controls, as indicated by the decline in toluene:dodecane ratio (Fig. 3). No noticeable change was observed in the ratios of other components of the heavy condensate. No sulfate depletion occurred in any of the sulfate-amended, anoxic sand microcosms.

Significant utilization of sulfate was also observed in the field *(4,5)*. Ground water samples collected from a zone upgradient of the contamination, representing background levels, indicated sulfate concentrations on the order of 230 mg/L, whereas those collected from the contaminated region near the top of the water table showed no sulfate. Also, visual inspection of soil cores showed a significant accumulation of a black precipitate (acid-volatile iron sulfide) associated solely with hydrocarbon contamination. Sulfate depletion was, therefore, linked to the presence of hydrocarbon. The microcosm experiments also indicate the potential for sulfate reduction as a possible mechanism for hydrocarbon remediation at the Denver basin field sites.

Hydrocarbon biodegradation linked to nitrate or iron reduction was not demonstrated in this study. Nitrate consumption was observed in biotic, nitrate-amended soil microcosms (Fig. 4), but not in biotic, nitrate-amended sand

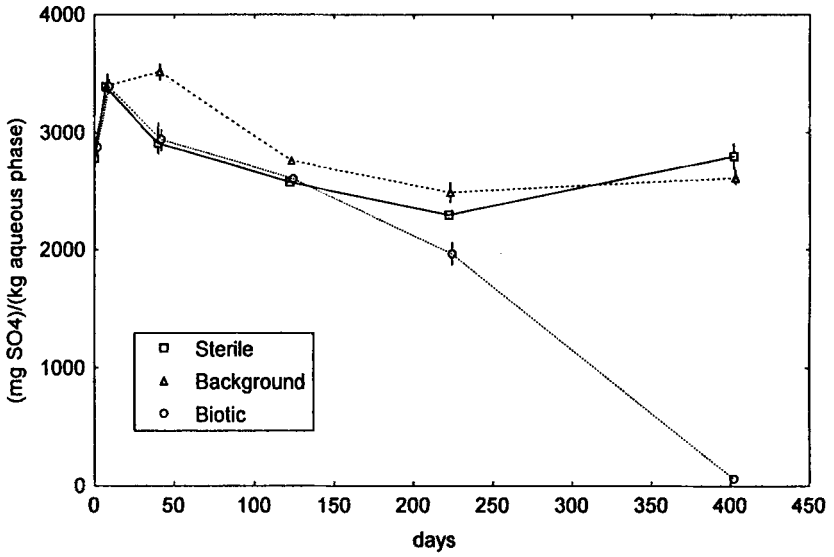

Fig. 2. Sulfate reduction in anoxic soil microcosms with exogenous sulfate added.

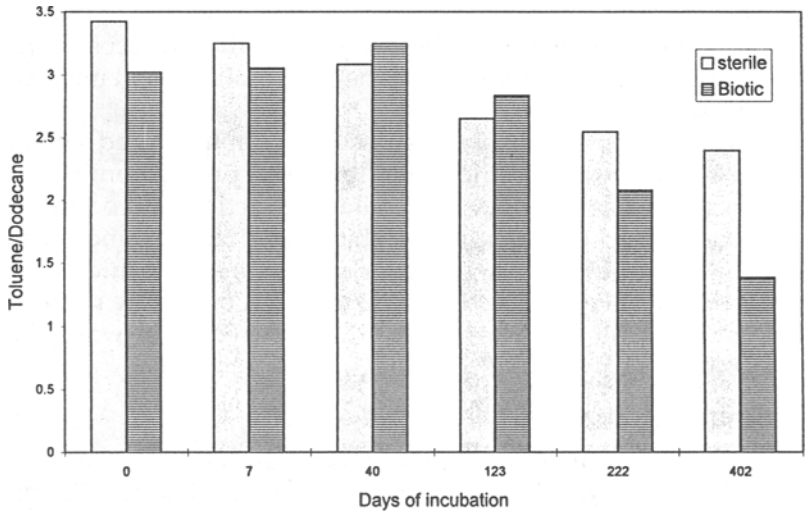

Fig. 3. Ratio of toluene to dodecane in anoxic, soil microcosms with exogenous sulfate added.

microcosms (data not shown). Nitrate consumption was also observed in the background microcosms, which contained no hydrocarbon, at a rate similar to that seen in biotic microcosms (Fig. 4). These data indicate that the organic carbon in the soil rather than the hydrocarbon served as the electron donor. Native soil contained about 1000 mg, and the soil inoculum contained about 900 mg of organic carbon/kg (dry wt) of soil (Leco Furnace test).

Production of Fe(II) was observed in Fe(III)-amended microcosms with and without hydrocarbon under anoxic conditions, which suggests that Fe(III) reduction was linked to oxidation of background carbon in the soil rather than oxidation

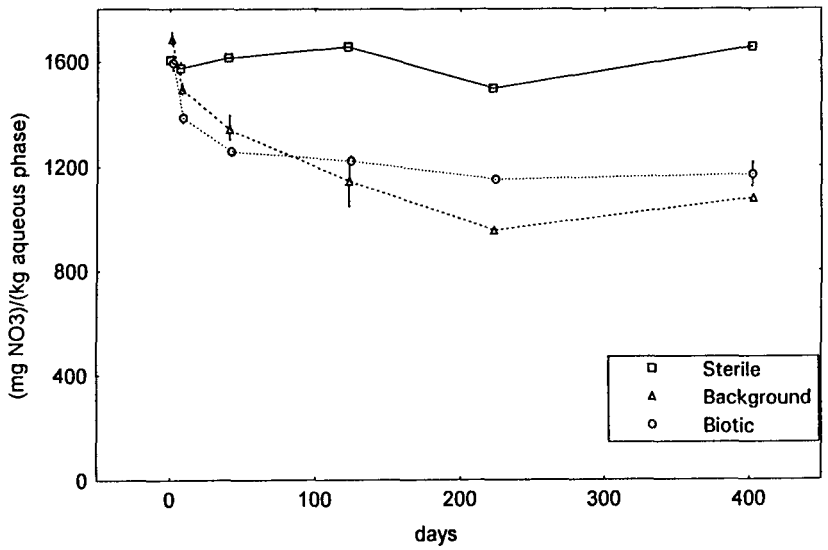

Fig. 4. Nitrate use in anoxic, soil microcosms with exogenous nitrate added.

of added hydrocarbon. A corresponding decrease in Fe(III) was not observed. Therefore, a mass balance could not be closed on iron in the microcosm. No change in the Fe(II)- to Fe(III)-ratio was observed in the sterile, anoxic soil microcosms, or any of the anoxic, sand microcosms.

In microcosms without an exogenous electron acceptor added, consumption of indigenous nitrate occurred first followed by Fe(II) production, regardless of whether hydrocarbon was present or not. Sulfate reduction also occurred simultaneously with the Fe(II) production in both biotic and background microcosms. The amount of sulfate reduced was, however, greater in biotic microcosms than in background soil microcosms. No Fe(II) production or sulfate reduction was observed in the anoxic, sand microcosms. Again, sulfate reduction was linked to the presence of hydrocarbon.

Oxygen-Limited Conditions

Experimental results suggest that nitrate may have been used as an electron acceptor for hydrocarbon biodegradation under oxygen-limited conditions. About 1200 mg/kg of nitrate was reduced in biotic, nitrate-amended soil microcosms with hydrocarbon, and little nitrate loss was observed in soil microcosms without hydrocarbon added or in sterile controls (Fig. 5). About 500 mg/kg of nitrate were used in biotic, nitrate-amended sand microcosms. Since the amount of nonhydrocarbon organic matter was very low in sand microcosms, this supports the conclusion that nitrate reduction was linked to hydrocarbon use. No nitrate use was observed in sterile, sand microcosms with hydrocarbon or in sand microcosms without hydrocarbon added.

The amount of oxygen present initially in oxygen-limited microcosms was sufficient for complete mineralization of only 1.2% of the initial TPH present. The presence of a limited amount of oxygen may have produced partially oxygenated intermediates, which were more amenable to further oxidation with nitrate than the original hydrocarbon.

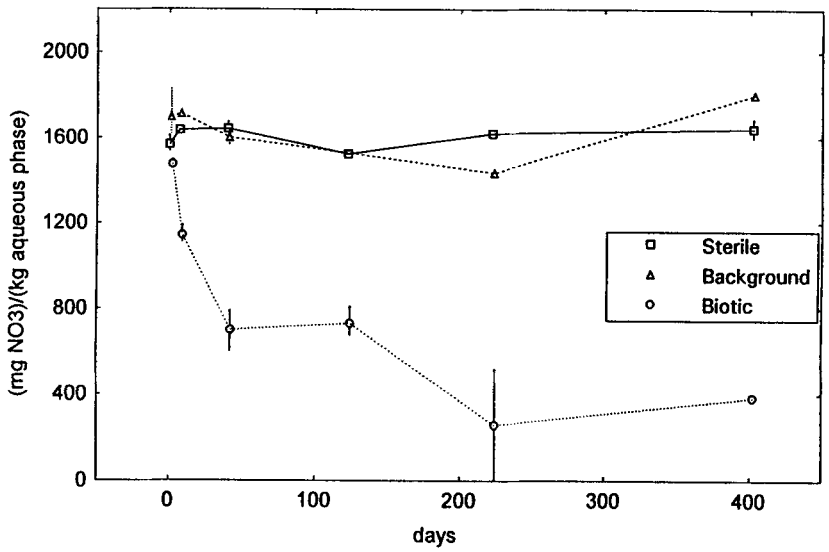

Fig. 5. Nitrate use in oxygen-limited, soil microcosms with exogenous nitrate added.

Sulfate reduction in the oxygen-limited sulfate-amended microcosms was similar to that observed in anoxic microcosms with strong evidence suggesting a linkage between sulfate reduction and bio-oxidation of condensate hydrocarbons. Sulfate reduction started only after a lag period of 222 d, which was longer than that observed in the corresponding anoxic experiment (lag of 122 d). About 700 mg/kg (aqueous phase) of sulfate were reduced in the sand microcosms and more than 2000 mg/(kg aqueous phase) of sulfate reduction were observed in the biotic, soil microcosms, both of which contained hydrocarbon. No sulfate reduction was observed in soil or sand microcosms that lacked hydrocarbon, or in sterile controls.

Fe(II) production was also observed after the 222-d lag period in the biotic microcosms amended with sulfate and hydrocarbon. The ratio of Fe(II)/Fe(III) increased from about 0.5 to 15.0 and from 1.0 to 10.0 in soil and sand microcosms, respectively. Fe(II) production may have resulted from chemical reduction of Fe(III) by the sulfide produced by microbial sulfate reduction *(10)*.

In oxygen-limited microcosms with Fe(III) added as the electron acceptor, Fe(II) production and Fe(III) consumption were observed in the soil and sand, biotic microcosms with hydrocarbon added. The Fe(II)/Fe(lII) ratio increased from about 0.5 to 3.0 and from 0.5 to 6 in 402 d in the biotic, soil and sand microcosms, respectively. No Fe(II) production or Fe(III) reduction occurred in the background soil, sand microcosms, or sterile controls. This suggests that the presence of a limited amount of oxygen in the microcosms enhanced the utilization of Fe(III) as an electron acceptor, most probably by producing partially oxygenated intermediates from the hydrocarbons. Sulfate reduction was observed in the soil microcosms; however, the low amounts of sulfate in the sand microcosms preclude a similar conclusion in these microcosms.

Acetate accumulation was observed in the biotic, oxygen-limited sand microcosms with hydrocarbon added; however, no acetate production was seen in soil microcosms. Acetate concentration reached a peak of 400 mg/kg (aqueous phase) at 123 d and then declined to zero in 402 d.

In microcosms without an exogenous electron acceptor added, nitrate depletion followed by simultaneous Fe(II) production and sulfate reduction occurred in the oxygen-limited, biotic microcosms. No change was observed in the concentrations of electron acceptors in the background microcosms that lacked hydrocarbon or sterile controls. The Fe(II)/Fe(III) ratio increased from 0.4 to 3.0 and from 1.1 to 4.8 in 402 d in the biotic, soil and sand microcosms amended with hydrocarbon, respectively.

CONCLUSIONS

Sulfate reduction was stimulated in the presence of gas condensate hydrocarbons under both anoxic and oxygen-limited conditions. A corresponding decrease in toluene was observed in the biotic, anoxic, and oxygen-limited soil microcosms concomitantly with sulfate reduction. Field observations show the depletion of sulfate from soil and ground water in contaminated areas *(4)*. Nitrate and Fe(III) reduction occurred in anoxic microcosms; however, these could not be linked directly to hydrocarbon biodegradation owing to the presence of an alternate carbon source in the soil. Nitrate was observed to inhibit microbial iron reduction; however, sulfate reduction occurred simultaneously with iron reduction.

Greater consumption of nitrate and Fe(III) were observed under oxygen-limited conditions in the presence of added hydrocarbon. This may be owing to partial oxidation of hydrocarbons with the limited amount of oxygen present as the electron acceptor.

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