0273-2289/97/63–65—0719$11.00

The Potential for Intrinsic Bioremediation of BTEX Hydrocarbons in Soil/Ground Water Contaminated with Gas Condensate

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ABSTRACT

Gas condensate liquids contaminate soil and ground water at two gas production sites in the Denver Basin, CO. A detailed field study was carried out at these sites to determine the applicability of intrinsic bioremediation as a remediation option. Ground water monitoring at the field sites and analysis of soil cores suggested that intrinsic bioremediation is occurring at the sites by multiple pathways, including aerobic oxidation, sulfate reduction, and possibly reduction Fe(III) reduction.

Laboratory investigations were conducted to verify that the water-soluble components of the gas condensate (benzene, toluene, ethylbenzene, and xylene [BTEX]) are intrinsically biodegradable under anoxic conditions in the presence of alternate electron acceptors and soil from the field site. Slurry-phase experiments were conducted in which soil obtained from the field site was mixed with an aqueous phase containing nutrients and electron acceptors (nitrate, Fe[III], sulfate and carbon dioxide) in serum bottles. The aqueous phase also contained soluble components of gas condensate, at two different hydrocarbon concentrations, obtained from the field site. The soil was either pristine (native) soil or soil obtained from a condensate-contaminated region. The aqueous

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phase was sampled for electron acceptors, hydrocarbons, and possible products of hydrocarbon degradation.

Toluene and xylenes were biodegraded with nitrate or sulfate as the electron acceptor. No degradation of benzene was observed under anoxic conditions.

Index Entries: Intrinsic bioremediation; gas condensate; anoxic; BTEX; hydrocarbon; sulfate reduction; denitrification.

INTRODUCTION

Soil and ground water contamination by petroleum hydrocarbons is an ongoing environmental problem. The BTEX compounds 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 anoxic 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 *(2–6)*.

Gas condensate liquids contaminate the soil and ground water at certain 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 anoxic bioremediation of hydrocarbons occurs at a sufficient rate and to an 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 have suggested that intrinsic bioremediation is occurring at the contaminated sites by multiple pathways, including aerobic oxidation, sulfate reduction, and possibly Fe(III) reduction *(7,8)*.

Laboratory investigations have been conducted to accompany field observations in order to verify hydrocarbon degradation by field organisms and identify the primary anoxic biodegradation mechanisms. Two types of experiments were conducted, saturated soil studies with excess hydrocarbon (free phase) and limiting amounts of electron acceptors, and slurry experiments that were hydrocarbon-limiting. The details of the saturated soil experiment are given elsewhere *(9)*. Sulfate reduction was found to be the major mechanism of hydrocarbon degradation under anoxic conditions in the presence of a free hydrocarbon phase. Nitrate and Fe(III) reduction was also observed in the anoxic saturated soil experiments; however, these could not be linked to hydrocarbon degradation. In the presence of an initial limited amount of oxygen, nitrate and Fe(III) reduction was observed and could be linked to the presence of hydrocarbon in the microcosms. It was proposed that utilization of these alternate

electron acceptors was stimulated by limited oxygen by generating partially oxygenated hydrocarbons, which were better substrates for anoxic degradation mechanisms.

The slurry-phase, hydrocarbon-limited experiments were conducted to determine which individual water-soluble components of the gas condensate were intrinsically biodegradable under anoxic conditions in the presence of alternate electron acceptors and soil from the field site. Four different electron acceptor conditions were investigated: nitrate, sulfate, Fe(III), and carbon dioxide (methanogenic). The conditions in these experiments simulated the conditions found in the ground water plume down gradient of the residual-free hydrocarbon phase.

MATERIALS AND METHODS

Composition of Slurry-Phase Microcosms

Slurry-phase experiments were carried out in 160-mL serum bottles with 40 g of soil and 80 g of aqueous phase. Microcosms were prepared with two types of soil, one obtained from a condensate-contaminated region and the other obtained from an uncontaminated region (native soil) from the field site. A mineralogical analysis of the native and contaminated soil from these sites has been given previously *(9)*. The native soil was predried for 24 h at 70°C to facilitate handling and sieved through a standard 10-mesh sieve before use. The contaminated soil was collected, transported, and stored anaerobically, and was processed only in an oxygen-free atmosphere in the anaerobic chamber. The contaminated soil contained approx 1000 mg/kg (dry basis) total petroleum hydrocarbons (TPH). These hydrocarbons were shown by gas chromatography to be similar to the gas condensate produced at the site except for reduced concentrations of lighter hydrocarbons. Each microcosm containing contaminated soil, therefore, contained about 40 mg TPH, which could be expected to be significantly depleted in water-soluble components.

The liquid phase consisted of a base medium prepared in deionized water with 1 mL/100 mL trace metals solution + 2.0 mL/100 mL mineral salts solution and 0.35 g/100 mL sodium bicarbonate. The composition of the trace metals solution and mineral stock solution is given by Tanner et al. *(10)*. A low concentration (0.0001%) of resazurin was added to the medium to indicate oxidation-reduction potentials above –0.042 V *(11)*. Resazurin changes color from blue (aerobic) to pink (partially anoxic) at a redox potential of about 0.78 V. This change of color is irreversible *(12)*. The indicator also changes color from pink to colorless (anaerobic) at about –0.042 V. This change is reversible and is generally used to indicate an anaerobic condition.

Nitrate, Fe(III), sulfate, and carbon dioxide were added to microcosms as electron acceptors. The carbon dioxide was present in all micro-

cosms as a part of the buffering system and was, therefore, a potential electron acceptor in all microcosms. The amount of electron acceptor added in each case was in excess of that required for complete mineralization of the hydrocarbon to carbon dioxide. Nitrate and sulfate were added as their sodium salts to the base medium to obtain nutrient medium for the nitrate-amended and sulfate-amended experiments, respectively. Fe(III) was added in the form of an amorphous Fe(III) oxyhydroxide gel. The concentrations of the electron acceptors were as follows: 19.0 m*M* nitrate in nitrate-amended, 80.0 m*M* Fe(III) in Fe(III)-amended, and 15.6 m*M* sulfate in sulfate-amended microcosms. All microcosms contained 20.0% carbon dioxide in the headspace.

The complete microcosms contained soluble components of the gas condensate and were prepared as follows. To simulate the actual contaminant found in the contaminated region, the light hydrocarbons were partially volatilized by heating the gas condensate at 105°C for 1 h to remove about 20 wt% of the hydrocarbon. The heavier retained fraction was referred to as the heavy condensate. The base media amended with electron acceptors (except for the Fe[III]-amended set, where the Fe[III] gel was added directly to microcosms) were contacted with the heavy condensate for a period of 24 h. The organic phase was then separated, giving an aqueous phase saturated with the soluble components of the heavy condensate (approx 32.0 mg/kg total BTEX). The individual components were at the following concentrations in the saturated medium: 6.5 mg/kg benzene, 18.0 mg/kg toluene, 0.4 mg/kg ethylbenzene, 6.0 mg/kg *m,p*-xylene, and 1.0 mg/kg *o*-xylene. The microcosms were prepared with two different total hydrocarbon concentrations, one with 100% hydrocarbon saturation (prepared as given above) and the other with 60% saturation. In the first set of microcosms (100% dissolved hydrocarbon saturation), 80 g of the heavy condensate-saturated solution was used, and in a second set (60% dissolved hydrocarbon saturation), 48 g of the condensate-contacted medium was mixed with 32 g of fresh base medium + electron acceptors resulting in 60% hydrocarbon saturation (approx 19.2 mg/kg BTEX). The microcosms containing 100% dissolved hydrocarbon saturation are referred as "high-BTEX" microcosms, and those with 60% dissolved hydrocarbon saturation are referred as "low-BTEX" microcosms.

Cysteine sulfide (reducing agent) was added to sulfate-amended microcosms to lower the redox potential in the microcosms (1.6 m*M* sulfide, 0.33 m*M* cysteine). No cysteine sulfide was added to any other microcosms. All operations were carried out inside an anaerobic chamber. The serum bottles were then stoppered with the Teflon-lined, black, butyl-rubber composite stoppers, removed from the chamber, and immediately crimped with aluminum crimp caps. The headspace in the microcosms was 20% CO_2 + bal N_2.

Sterile controls were prepared in addition to biotic microcosms in order to account for any abiotic losses. Sterile controls were prepared

Table 1
Details of Design of the Slurry-Phase Experiment

soil types	2 (native-soil, contaminated-soil)
microcosm type	2 (biotic, sterile)
carbon source	soluble hydrocarbons, 2 concentrations, 100% saturation of aqueous phase with condensate (32 mg/kg BTEX), and 60% saturation, (19.2 mg/kg BTEX)
terminal electron acceptors	4 (nitrate, Fe(III), sulfate, carbon dioxide)
temperature	1 (30°C)
replicates	2

exactly the same way as the biotic microcosms, except that they were sterilized at 121°C for 60 min. After sterilization, the microcosms were cooled to room temperature and then transferred into an incubator. All microcosms were prepared in duplicate and incubated for 160–200 d at 30°C.

Analysis of samples taken after 15 d of incubation from the nitrate-amended microcosms showed about 90% depletion of toluene in biotic, contaminated-soil microcosms. These microcosms were replenished at this time by replacing about 80% of the liquid phase from the microcosms with fresh medium. Data are reported for these microcosms from this time forward (*see below*). Analysis of all microcosms was done by periodic sampling of the aqueous phase using an anaerobic, sterile syringe and needle. The sampling methodology is given by Borole *(13)*. Details of the design of slurry-phase experiments are given in Table 1.

Analytical

Monoaromatic hydrocarbons (BTEX) were analyzed on an On-Column HP 5890 gas chromatograph with a flame ionization detector. A 15-m long DB-1 megabore (0.32 mm id) capillary column with 1-μ film thickness was used to carry out the separation. Column conditions were as follows: prehold at 35°C for 1 min; oven temperature, 35–170°C; program rate, 7°C/min, "oven track" ON with a temperature difference of 3°C between the oven and injector; detector temperature, 340°C. Calibration standards for the BTEX compounds were made by dissolving pure compounds in a 1:1 (vol:vol) methanol:water. The methods used for analysis of anions and straight-chain organic acids are given elsewhere *(9)*.

RESULTS AND DISCUSSION

Nitrate Amended

Nitrate utilization and degradation of hydrocarbons (primarily toluene and *o*-xylene) were observed in the biotic microcosms with both contaminated and native soil (Figs. 1 and 2). Although there was little difference in the amount of nitrate reduced or in the removal of toluene or *o*-xylene in microcosms with contaminated or native soil at the lower BTEX concentration (Fig. 1), significant differences were observed at the higher BTEX concentration (Fig. 2). At the higher BTEX concentration, toluene depletion was incomplete and nitrate utilization lower in the contaminated-soil microcosms, suggesting inhibition of toluene utilization possibly owing to accumulation of an inhibitory product. Alternatively, depletion of a nutrient in the contaminated soil possibly made utilization of the greater concentration of hydrocarbon impossible, or there may have been a combination of nutrient limitation, product inhibition, and inhibition resulting from elevated hydrocarbon concentrations.

o-Xylene was also observed to be utilized in contaminated-soil microcosms at lower BTEX concentrations; however, at the higher BTEX concentration, *o*-xylene utilization was not complete. Complete *o*-xylene degradation was observed in both the low- and high-BTEX biotic, native-soil microcosms. Some biodegradation of *m,p*-xylene was observed in the microcosms with native soil at higher BTEX concentration; however, no *m,p*-xylene depletion occurred in high-BTEX, contaminated-soil microcosms. No degradation of benzene or ethylbenzene was observed in any nitrate-amended microcosms.

The results given in Figs. 1 and 2 for microcosms with contaminated soil are those in which the aqueous medium was replenished with 80% fresh medium. In the original high-BTEX microcosms, 90% depletion of toluene and 60% depletion of *o*-xylene was observed in first 15 d; however, in the replenished microcosms, only 50% toluene depletion occurred, and no *o*-xylene depletion was observed. Depletion of toluene and *o*-xylene was biologically mediated, since the sterile microcosms did not show a similar decrease. The microcosms prepared with native soil were not replenished, and the results presented in Figs. 1 and 2 are for a first-time exposure to BTEX·Toluene depletion occurred at both the high- and low-BTEX concentrations with no apparent lag at the lower BTEX concentration, but with a lag of about 10 d at the higher BTEX concentration. These observations are again consistent with a nutrient limitation or inhibitory substance associated with the contaminated soil.

The contaminated soil contained black precipitates of iron sulfide produced by sulfate reduction in the field. Sulfate production was observed in nitrate-amended, contaminated-soil microcosms. This production was most likely biologically mediated under denitrifying condi-

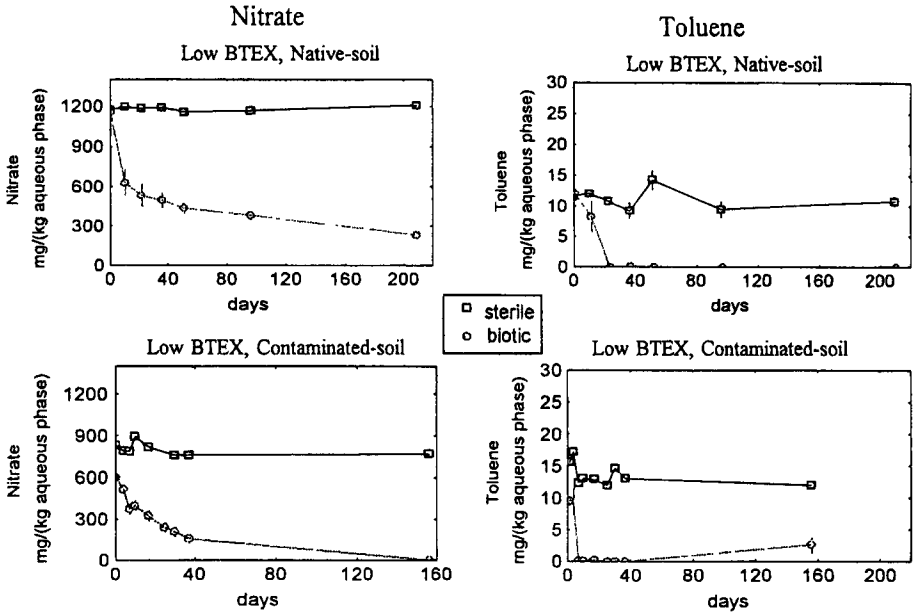

Fig. 1. Nitrate reduction and toluene depletion in low-BTEX, nitrate-amended slurry-phase microcosms. (Vertical bars indicate range of duplicate samples.)

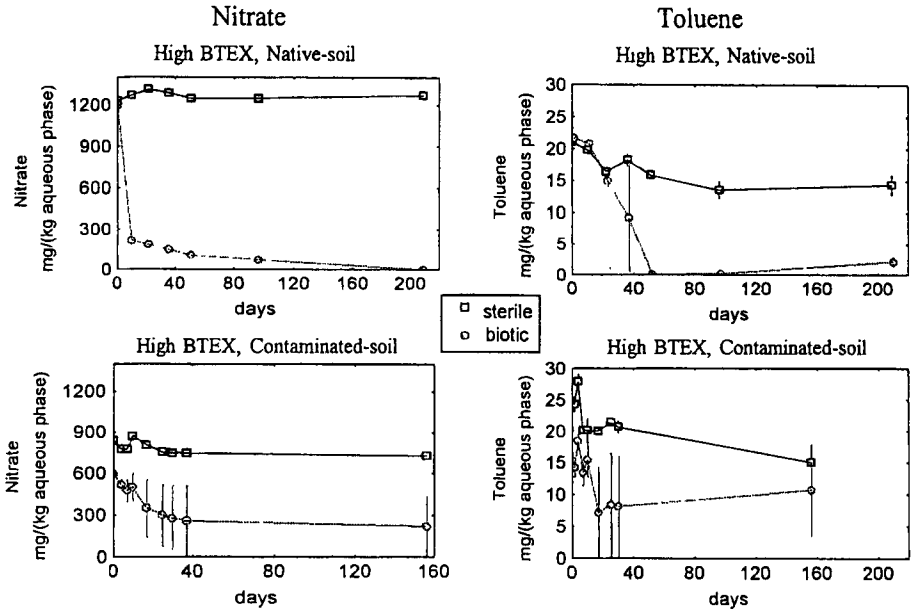

Fig. 2. Nitrate reduction and toluene depletion in high-BTEX, nitrate-amended, slurry-phase microcosms. (Vertical bars indicate range of duplicate samples.)

Table 2
Comparison of Stoichiometric and Observed Utilization of Nitrate as Electron Acceptor for Toluene and *o*-Xylene Degradation

Microcosm type	NO_3 used/ (toluene + o-xylene) (wt/wt)	
	predicted	observed
Low BTEX, contaminated-soil microcosms	5.04	32.5
High BTEX, contaminated-soil microcosms	5.04	6.17
Low BTEX, native-soil microcosms	5.04	73.6
High BTEX, native-soil microcosms	5.04	48.4

tions, since only biotic microcosms showed sulfate production. The ratio of nitrate consumed to total initial toluene and *o*-xylene is given in Table 2 for each nitrate-amended microcosms. Nitrate consumed was corrected for nitrate utilized for sulfide oxidation based on sulfate produced.

The stoichiometry for complete degradation of toluene with nitrate as the electron acceptor is given by Eq. (1) *(4)*.

$$C_7H_8 + 7.2\,H^+ + 7.2\,NO_3^- \Rightarrow 7\,CO_2 + 7.6\,H_2O + 3.6\,N_2 \qquad (1)$$

The higher utilization of nitrate observed in these experiments may be owing to (1) incomplete reduction of nitrate *(14)* or (2) increased electron acceptor demand resulting from indigenous organic carbon present in the soil (1000 mg total organic carbon/kg dry soil). Accumulation of nitrite was observed in both native-soil and contaminated-soil biotic microcosms; however, all the nitrite produced was depleted before the end of the experiment. The amount of nitrite produced in biotic, native-soil microcosms was different from that produced in the corresponding contaminated-soil microcosms. Nitrite concentration reached a peak of about 120 mg/(kg aqueous phase) in low-BTEX and 360 mg/(kg aqueous phase) in high-BTEX biotic, native-soil microcosms. In the contaminated-soil microcosms, the corresponding concentrations were 80 mg/(kg aqueous phase) and 70 mg/(kg aqueous phase), respectively. The peak in nitrite concentration occurred in the first 10 d with both soils; however, no BTEX consumption occurred during this period, which suggests that an alternate carbon source (indigenous organic carbon) may have served as the carbon donor during this time. Moreover, since much larger amounts of nitrite were used and more nitrite was produced in native-soil microcosms, the indigenous organic carbon

present in the native soil is inferred to be more readily degradable under denitrifying conditions than that present in contaminated soil (principally hydrocarbon).

Sulfate Amended

Sulfate utilization was observed in both native-soil and contaminated-soil biotic microcosms (Figs. 3 and 4); however, longer lag times were observed with native-soil (100 d) compared to contaminated-soil microcosms (15–37 d). The rate of sulfate depletion was faster and more sulfate was consumed in contaminated-soil microcosms compared to the native-soil microcosms. Sulfate reduction activity was observed in the plume region of the Denver Basin contaminated site *(7)*, therefore, the contaminated soil was expected to be enriched in sulfate-reducing bacteria (SRB). These results suggest that this was the case.

In the contaminated-soil microcosms, toluene depletion accompanied sulfate utilization at the lower and higher BTEX concentrations. However, at the higher BTEX concentration, the two replicates showed appreciable differences in toluene concentrations during the experiment. At both BTEX concentrations, toluene was completely depleted within 60 d (low BTEX) and 100 d (high BTEX). Utilization of xylenes was also observed in microcosms with contaminated soil (Table 3). In native-soil microcosms, utilization of toluene or xylenes was less conclusive, and less sulfate was consumed compared to corresponding microcosms with contaminated soil. Utilization of ethylbenzene was inconclusive at lower BTEX concentrations, but ethylbenzene utilization was documented at the higher BTEX concentration in both native-soil and contaminated-soil microcosms (Table 3). No utilization of benzene was observed in any sulfate-amended microcosms.

Toluene mineralization to CO_2 with sulfate as the electron acceptor (with no cell mass production) is given by Eq. (2) *(2)*

$$C_7H_8 + 4.5\ SO_4^- + 3\ H_2O \Rightarrow 7\ HCO_3^- + 2.25\ HS^- + 2.25\ H_2S + 0.25\ H^+ \quad (2)$$

The amount of sulfate utilized in these microcosms was far in excess of that required to fully oxidize all of the BTEX in the microcosms. The indigenous organic carbon present in native soil and contaminated soil was capable of producing a sulfate demand, and therefore, part of the sulfate consumed may have been used for oxidation of the indigenous organic carbon. Large amounts of acetate (200 mg/kg aqueous phase) were observed in both low-BTEX and high-BTEX sulfate-amended microcosms in both native-soil and contaminated-soil microcosms during the first 15 d. No detectable sulfate reduction or BTEX depletion occurred during this initial time period, which implies that BTEX compounds did not serve as the electron donor for the production of acetate. No other straight- or branched-chain fatty acids were detected in the aqueous phase of any microcosms.

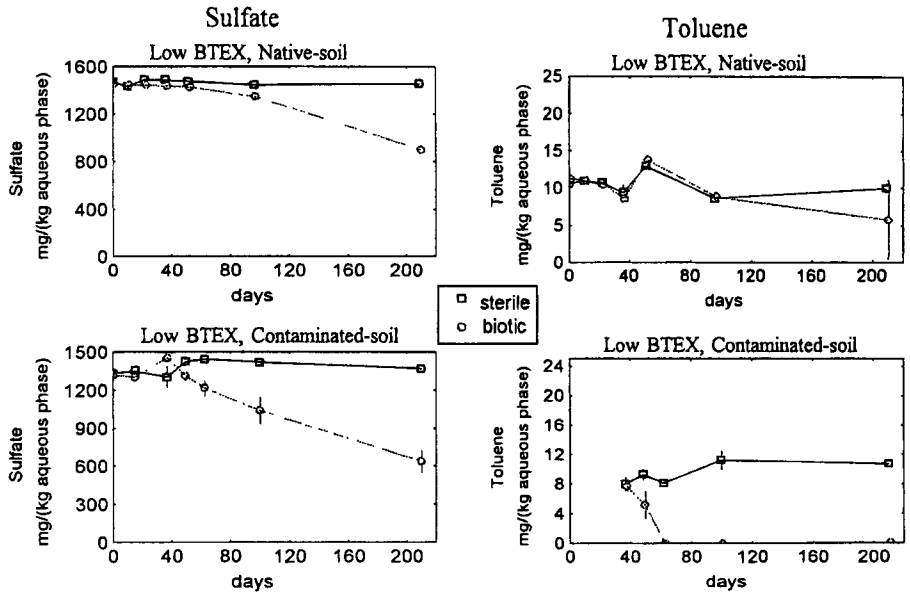

Fig. 3. Sulfate reduction and toluene depletion in low-BTEX, sulfate-amended, slurry-phase microcosms. (Vertical bars indicate range of duplicate samples.)

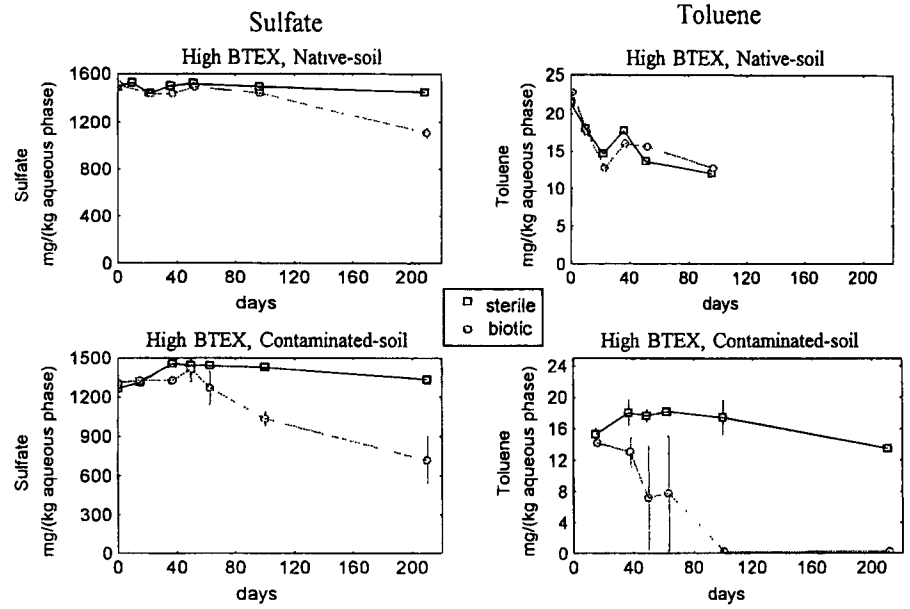

Fig. 4. Sulfate reduction and toluene depletion in high-BTEX, sulfate-amended, slurry-phase microcosms. (Vertical bars indicate range of duplicate samples.)

Table 3
Qualitative Analysis of Biotic and Abiotic Losses of BTEX in Slurry-Phase Microcosms

Anoxic	Soil type	BTEX amount	Benzene	Toluene	Ethyl-benzene	m,p-Xylene	o-Xylene
			Biodegradation - Abiotic loss - lag period (days)*				
NO_3^-	Native	low	N-N	Y-N-0	?-N	?-Y	Y-Y-0
	Native	high	N-Y	Y-Y-4	?-N	Y-Y-10	Y-Y-0
	Contaminated	low	N-N	Y-Y-7	?-N	N-Y-7	Y-N-4
	Contaminated	high	N-N	Y-Y-10	N-Y	N-Y	?-Y
Fe(III)	Native	low	N-N	Y-N-0	N-N	N-Y	?-Y
	Native	high	N-Y	Y-Y-0	N-N	N-Y	N-Y
	Contaminated	low	N-N	N	N-N	?-Y	Y-?-50
	Contaminated	high	N-?	Y-?-100	Y-?-62	Y-Y-15	Y-?-50
SO_4^{--}	Native	low	N-N	Y-N-100	?-N	Y-Y	Y-Y-10
	Native	high	N-Y	N-N-100	Y-N	Y-Y	Y-Y-0
	Contaminated	low	N-?	Y-?-37	N-N	Y-?-37	Y-?-15
	Contaminated	high	N-?	Y-?-15	Y-Y-62	Y-Y-0	Y-Y-15
Non-amended	Native	low	N-N	N-N	N-Y	N-Y	N-Y
	Native	high	N-Y	N-Y	Y-Y-22	Y-Y	?-Y
	Contaminated	low	N-?	N-N	N-?	N-Y	N-N
	Contaminated	high	N-Y	Y-Y-100	N-Y	N-Y	N-Y

* The 2 letters N and Y stand for no and yes, respectively, and indicate whether biodegradation or abiotic loss took place in the microcosms. A question mark implies unreliable or nonconclusive data. A third numerical character is used in those cases where biodegradation was observed to indicate the lag time (days) observed for biodegradation of the BTEX component.

Fe(III) Amended and Nonamended

Iron analysis could not be done in this experiment because of the nonsacrificial nature of the microcosms, and no analysis was conducted on the liquid phase since a negligible amount of iron was present in the aqueous phase. No benzene depletion occurred in any of the contaminated-soil or native-soil microcosms. Toluene depletion was observed in native-soil microcosms and not in contaminated-soil microcosms amended with Fe(III). Degradation of xylenes was observed in contaminated-soil microcosms, but not in native-soil microcosms amended with Fe(III) (data not shown).

Benzene or toluene biodegradation did not occur in any of the nonamended microcosms. Only some abiotic loss of xylenes was observed in the nonamended contaminated-soil and native-soil microcosms. No attempt was made to analyze for methane in the nonamended microcosms, since sulfate (present as a soil constituent) was also present until the last time event (210 d), and degradation of BTEX components could not be documented.

CONCLUSIONS

Biodegradation of toluene and xylenes has been verified under sulfate-reducing and denitrifying conditions in microcosms with either native soil or soil previously exposed to gas condensate (contaminated soil) from a Denver Basin gas-producing field site as a source of microorganisms. These experiments confirmed sulfate reduction and denitrification as viable anoxic mechanisms for the degradation of compounds in ground water contaminated with gas condensate. Since field observations indicate nitrate is limited at the site relative to sulfate, sulfate can be expected to be the dominant of the two mechanisms in the field. Depletion of BTEX hydrocarbons could not be linked to Fe(III) reduction or methanogenesis in these experiments.

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