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ENVIRONMENTAL RESEARCH BRIEF

Anaerobic Biodegradation of BTEX in Aquifer Material

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Abstract

Laboratory and field experiments were conducted in two petroleum-contaminated aquifers to examine the anaerobic biodegradation of benzene, toluene, ethylbenzene and xylene isomers (BTEX) under ambient conditions. At both sites, destructive microcosm experiments were conducted following the EPA protocol for estimation of anaerobic microbiological transformation rate data (*Federal Register,* Vol. 53, No. 115). Aquifer material was collected from locations at the source, mid-plume and end-plume at both sites, incubated under ambient conditions, and monitored for disappearance of the test compounds. In the mid-plume location at the second site, in-situ column experiments were also conducted for comparison with the laboratory microcosm and field-scale results.

In material from the first site, collected from a location in the Sleeping Bear Dunes National Lakeshore, Michigan, toluene biodegraded in microcosms under methanogenic conditions after a 60- to 246-day lag period. There was no statistically significant evidence of benzene, ethylbenzene, or xylene biodegradation in the microcosm study.

At the second site, near Rocky Point, North Carolina, all BTEX components biodegraded under ambient, anaerobic conditions. In the mid-plume microcosms, *m*-xylene biodegraded first followed by toluene, *o*-xylene and benzene under iron reducing conditions. None of the compounds biodegraded in the source area microcosms. In the end-plume microcosms, biodegradation was variable with extensive biodegradation in some microcosms and little or no biodegradaion in others. In all microcosm sets where biodegradation was measured, the compound being investigated degraded to a low but detectable level (5 to 30 µg/Liter), after which biodegradation slowed or stopped. Biodegradation rates for *m*-xylene and benzene in in-situ columns from the mid-plume location were similar to microcosm rates.

Anaerobic biodegradation of individual BTEX components often consisted of three distinct phases: (1) a lag period with little or no biodegradation; (2) a rapid degradation period; and (3) an asymptotic period where contaminant concentrations remained essentially constant. This pattern of biodegradation cannot be accurately described with a simple first-order decay function. In contrast to the behavior of the individual compounds, the biodegradation of total BTEX appears to more closely approximate a first-order decay function.

Introduction

This study focuses on the anaerobic biodegradation of benzene, toluene, ethylbenzene and xylene isomers (BTEX) in aquifer material from two petroleum-contaminated aquifers: Sleeping Bear Dunes National Lakeshore (SB) in Michigan and a site near Rocky Point, North Carolina (RP). The two sites examined were chosen because of their differences in plume size, contaminant

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residence time, geochemical environment and geologic setting. At both sites, previous field monitoring indicated that anaerobic biodegradation of one or more BTEX components was occurring (Borden*et al.*, 1995; Wilson*et al.*, 1994). Two different techniques were used to evaluate the ability of indigenous microorganisms to anaerobically degrade BTEX and to estimate the rate of degradation: (1) destructively sampled laboratory microcosms; and (2) in-situ test chambers. Both techniques consist of spiking aquifer sediment with BTEX and monitoring compound disappearance over time. Experimental conditions were designed to mimic ambient conditions in the aquifer to the maximum extent possible. Killed controls were monitored at the same time to differentiate between biological and abiotic losses.

Procedure

Laboratory microcosms were constructed with aquifer material from source, mid- and end-plume locations at both sites. The microcosms were prepared in serum bottles with a high sediment to water ratio of 1.8 dry g/mLiter. The experimental procedure was based on the EPA protocol for estimation of anaerobic microbiological transformation rate data (*Federal Register*, Vol. 53, No. 115). Multiple replicate microcosms were constructed using blended aquifer sediment and ground water recovered under anaerobic conditions. The aquifer sediment was collected aseptically and anaerobically using methods developed by U.S. EPA (Dunlap *et al.*, 1984). Ground water was collected anaerobically through a 0.45 micron filter from monitoring wells adjacent to each core location. Microcosms were spiked with approximately 2,000 µg/Liter of benzene, toluene, ethylbenzene, *o*-xylene and *m*-xylene and incubated in anaerobic containers stored at the ambient ground-water temperature, 16 °C. The microcosms were constructed in an anaerobic glove box using aseptic techniques. A reducing agent was added to microcosms constructed with SB sediment while naturally occurring Fe II (aq) served as a reducing agent in the RP microcosms. Resazurin was added to all microcosms and indicated anaerobic conditions throughout the monitoring period. Triplicate microcosms and abiotic controls were sacrificed at approximately monthly intervals for one to two years and analyzed to determine the loss of BTEX and changes in other electron acceptors and donors.

The in-situ columns were used at one location for comparison with the laboratory microcosms and are similar to the system used by Gillham *et al.* (1990). Each column consists of a chamber (1.0 m long) where sediment and ground water are isolated from the surrounding aquifer for controlled observation (Figure 1). Columns were installed by drilling a pilot hole and then installing a 15 cm-diameter by 3 m-long section of polyvinyl chloride (PVC) casing. Stainless steel tubing and 3 m of drill rod were attached to the equipment chamber. Argon was pumped into the casing to displace any oxygen present. The column was then pushed into the aquifer. Suction was applied to the stainless steel feed line to insure that the column was completely filled with aquifer material. The columns were then filled with anaerobic ground water containing BTEX. Two microcosm columns and one abiotic control were used in each experiment. The abiotic control columns were prepared by adding an inhibitor to the injection water (final concentration was either 0.1N HCL or 500 mg formaldehyde/Liter). All columns were monitored for BTEX, dissolved iron, sulfate, chloride, pH and dissolved oxygen (DO). Analytical methods followed in the microcosm and in-situ column experiments are described elsewhere (Hunt *et al.*, 1997; Beckman, 1994).

Effective first-order removal rates for BTEX in the microcosms and in-situ columns were estimated using the equation $C = Co \exp(-Kt)$, where K is the apparent first-order decay rate (day^{-1}), t is time and Co is the initial concentration. The biological loss rate was calculated as the difference between the loss rates in the microcosms and abiotic controls over the time period in which biological losses were observed. A two-tailed Student's test, assuming unequal variances, was performed to determine if the microcosm rates were statistically different from the abiotic control rates.

Results and Discussion Sleeping Bear Site

Site Characteristics

The first site (SB), located at the Sleeping Bear Dunes National Lakeshore, Michigan, is a highly transmissive glacial outwash consisting of coarse sand and gravel with calcium carbonate fragments. The soil has a relatively high organic carbon content (up to 2.2%) that primarily occurs as coatings on sand grains (>94%) (West *et al.*, 1994). The alkylbenzene plume covers a relatively short span (25 m) before it is intercepted by the Platte River. Contaminant residence time in the aquifer varies from 5 to 53 weeks because of fluctuations in the water table gradient. Oxygen, nitrate and sulfate are rapidly consumed, and dissolved iron is produced at the upgradient edge of the hydrocarbon spill. In the source area and downgradient contaminated aquifer, methane is produced and dissolved BTEX concentrations decline (Table 1). Field monitoring data indicate that toluene biodegrades

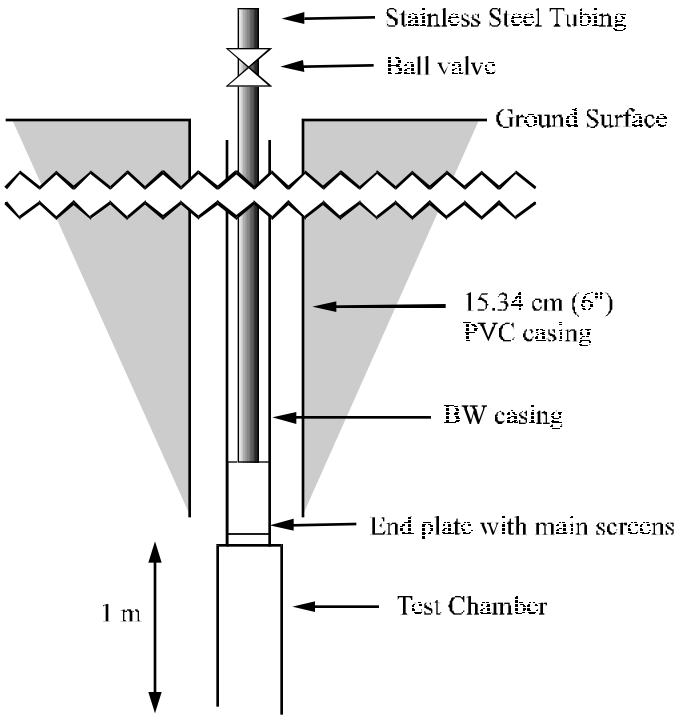

Figure 1. Schematic of in-situ test column.

TABLE 1. Geochemical Characterization of Ground Water at the SB and RP Sites

SB site

Parameter	Background	Source area	Mid-plume	End-plume
Distance from source (m)	-23	0	9.1	21.3
Well Screen (m)	2.7-4.7	2.7 - 3.7	1.2 - 2.1	0.6 - 1.5
Depth of Core (m)	NA[a]	2.3 - 4.2	0.5 - 2.4	0.6 - 2.3
Oxygen (mg/Liter) [b]	2.4	<0.1	<0.1	0.4
Nitrate (mg/Liter) [b]	67	2.2	0.3	0.1
Fe (aq, mg/Liter) [b]	3.5	7.7	4.1	5.2
Sulfate (mg/Liter) [b]	20.0	<0.05	6.0	<0.05
Methane (mg/Liter) [b]	0.08	0.10	1.7	3.1
Eh (mV) [c]	NM[d]	-294	-180	-186
pH [c]	NM	5.9	6.5	6.0
BTEX (mg/Liter) [c]	<0.001		3.3	2.0

RP site

Parameter	Background	Source area	Mid-plume	End-plume
Distance from source (m)	-55	0	183	327
Well Screen (m)	0.9-3.9	1.0-4.0	4.9-6.4	5.0-6.5
Depth of Core (m)	NA	4.3-5.0	3.8-4.8	3.8-4.6
Oxygen (mg/Liter) [e]	3.1	0.6	0.4	1.4
Nitrate (mg/Liter) [e]	19.6	<1.5	<1.5	<1.5
Fe (aq, mg/Liter) [e]	0.2	24.6	52.0	1.8
Sulfate (mg/Liter) [e]	18.9	34.8	4.0	12.8
Methane (mg/Liter) [e]	<0.001	0.096	0.096	0.40
Eh (mV) [e]	196	-132	-118	-187
pH [e]	4.6	5.8	6.1	7.1
Benzene (mg/Liter) [e]	<0.005	1.33	0.62	0.62
Toluene (mg/Liter) [e]	<0.005	10.4	0.083	0.028
Ethylbenzene (mg/Liter) [e]	<0.005	1.82	1.92	0.042
o-Xylene (mg/Liter) [e]	<0.005	3.88	0.042	0.010
m-, p-Xylene (mg/Liter) [e]	<0.005	8.18	2.75	0.34

[a]NA - not applicable

[b]Data are average of 2 samples taken over a 4-month period as reported by Wilson et al., 1994.

[c]Measured at time of core collection.

[d]NM - not measured.

[e]Data are average of 4 to 7 measurements taken over an 18-month period as reported by Gomez, 1993.

most rapidly with slower biodegradation of ethylbenzene and the xylene isomers (Wilson *et al.*, 1994). There was no evidence of anaerobic benzene degradation.

Microcosm Results

The rate and pattern of biodegradation were similar at all three locations at the SB site. Toluene biodegraded under methanogenic conditions at all three locations after a lag period that varied from 60 to 246 days. First-order decay rates during the period of active biodegradation were 0.042, 0.023 and 0.032 d^{-1} for the source, mid- and end-plume microcosms, respectively. Figure 2 shows the variation in dissolved toluene and methane in microcosms from the mid-plume location. The increase in methane greatly exceeded the amount that could be expected from the measured BTEX loss, indicating that other undefined substrates were being biotransformed. In addition, most of the methane was produced during the period when toluene was not biodegrading (Figure 2). Similar trends were measured in the source and end-plume microcosms. The aquifer material used in the microcosms contained ~0.6% organic carbon while West *et al.* (1994) found up to 2.2% organic carbon in aquifer sands 21 m upgradient of the plume. Acetate had also been detected in ground water from the site (Wilson *et al.*, 1994). Thus, background organic carbon was the major electron donor in the SB sediment from all three locations. The initiation of methane production prior to toluene biodegradation and the long lag times prior to the onset of toluene biodegradation may be due to the presence of more readily degradable substrates. In a

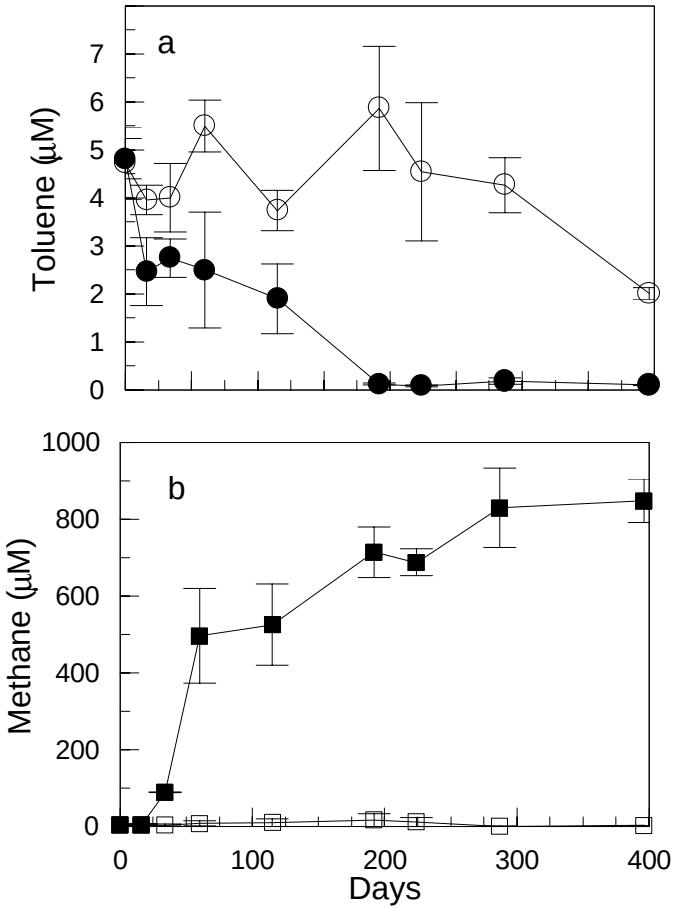

Figure 2. Average concentration of toluene (a) and methane (b) in microcosms (solid) and abiotic controls (open) from the mid-plume location at the SB site. Data are the average of three destructively sampled microcosms at each time point. Error bars represent one standard deviation.

study of methanogenic enrichment cultures derived from contaminated aquifer sediments, Edwards and Grbic-Galic (1994) found the presence of other organic substrates such as acetate, amino acids and propionate inhibited anaerobic degradation of toluene and *o*-xylene.

There was no evidence of *o*-xylene, *m*-xylene or benzene biodegradation at any location or ethylbenzene biodegradation in the source and mid-plume microcosms. However, for the end-plume microcosms, the ethylbenzene results are ambiguous. The first-order loss rate for ethylbenzene in the microcosms was not significantly different from the abiotic control over the entire test period; however, there was evidence of ethylbenzene degradation in selected replicates.

Wilson *et al*. (1994) reported biodegradation rates for BTEX components based on field monitoring of the SB plume coupled with estimates of contaminant retention time prior to discharge to the Platte River. Given the uncertainty in the calculation of both in-situ and microcosm biodegradation rates, quantitative comparison of these laboratory results and the field rate of Wilson *et al.* is inappropriate. Qualitative comparison of the field and laboratory data shows consistency with respect to: (1) the absence of benzene biodegradation; (2) the production of methane; and (3) the preferential biodegradation of toluene. While the laboratory data do not support or preclude low rates of ethylbenzene biodegradation, Wilson *et al*. (1994) reported biodegradation rates for ethylbenzene that were 10% of the rates reported for toluene.

Rocky Point Site

Site Characteristics

The second site (RP) is located on the coastal plain near Rocky Point, North Carolina, and is of marine origin. The site geology consists of dark gray and green micaceous fine sand, overlain by 1.5 to 4.5 m of silts, clays and clayey sands. The sediment is over 90% quartz sand with minor amounts of pyrite and muscovite flakes in a clay matrix. X-ray diffraction has shown that most of the iron in the aquifer solids is present as glauconite, an iron-rich clay mineral, with smaller amounts of the clays berthierine and possibly iron-rich illite (Becker, 1992).

Field monitoring (Table 1) indicates that toluene and *o*-xylene decline rapidly during transport through the first 100 m of the 300 m-long contaminant plume (Borden *et al*., 1995). Benzene and *m*-, *p*-xylene decline more slowly before discharging to a small drainage ditch. Anaerobic ethylbenzene biodegradation was not evident from the field data. At the upgradient edge of the plume, oxygen is rapidly depleted. Sulfate is depleted immediately downgradient of the contaminant source and dissolved Fe (II) increases in the mid-plume area, suggesting that biodegradation occurs using both sulfate and ferric iron as electron acceptors. The absence of significant methane in the monitoring wells indicates methanogenic fermentation is not a major process at this site.

The average ground-water velocity in the aquifer is 30 m/yr, resulting in a contaminant residence time in excess of 12 years (Borden *et al*., 1995).

Microcosm Results

At the RP site, anaerobic biodegradation of BTEX was a function of location within the contaminant plume. In the source area microcosms, none of the BTEX components degraded during 388 days of incubation. In the mid-plume microcosms, *m*-xylene biodegradation began with no lag, followed by toluene, *o*-xylene and benzene (Figure 3a). By day 140, both toluene and *o*-xylene had degraded to between 3 and 11 μg/Liter and then remained constant for the duration of the experiment. With the onset of toluene degradation after day 22, the rate of *m*-xylene loss declined and did not increase until toluene and *o*-xylene were below 22 μg/Liter at 120 days. Benzene degradation began after day 180, and by day 403 the benzene concentration was between 8 and 12 μg/Liter in each replicate (Figure 3b). Two isolated replicates in which BTEX degraded also exhibited ethylbenzene biodegradation during the last 100 days of incubation; however, the average decay rate for ethylbenzene was minimal over the sampling period.

In the mid-plume location, the dominant electron acceptor was ferric iron present as an iron-rich clay mineral, glauconite. Methane was not produced in any microcosm and manganese concentrations were below detection. While small amounts of sulfate were present initially, the dissolved sulfate concentration remained constant (Figure 3d) and was not sufficient to account for the observed BTEX loss. Dissolved iron concentrations varied but appeared to increase with time (Figure 3e). However, this increase was relatively small in proportion to the increase

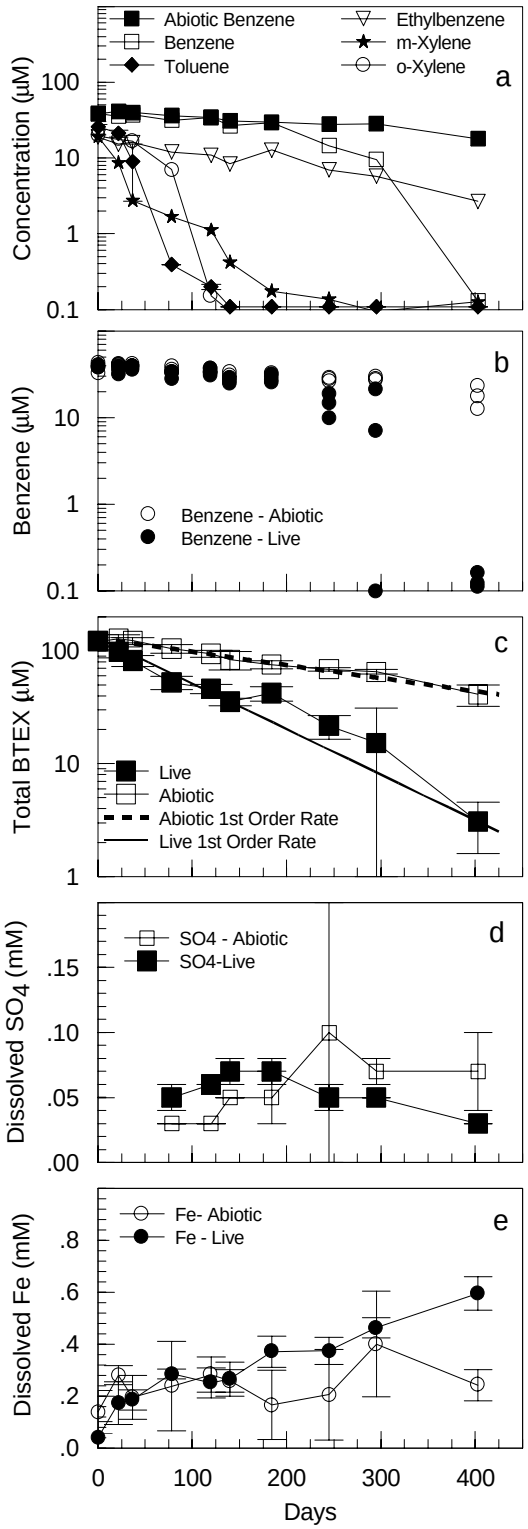

Figure 3. Results from mid-plume RP microcosms: (a) BTEX components, (b) benzene, (c) total BTEX, (d) dissolved SO_4^{-2}, and (e) dissolved Fe. Data in figures a, c, d and e are the average of three replicates destructively sampled at each time point. Error bars are ± one standard deviation. Abiotic benzene concentrations in Fgure 3a represent the behavior of the other BTEX components. Benzene data in Figure 3b are for individual microcosms.

in solid-phase Fe (II). At the end of the experiment, the measured increase in total Fe (II) was 200% of the amount predicted based on the measured loss of BTEX, assuming conversion to CO_2. This indicates that significant quantities of non-BTEX organic carbon also biodegraded under iron reducing conditions.

In the mid-plume microcosms, biodegradation of toluene, *o*-xylene and benzene consisted of three distinct phases: (1) a lag period with little or no biodegradation; (2) a rapid degradation period; and (3) an asymptotic period where contaminant concentrations remained essentially constant. This pattern of biodegradation cannot be accurately described with a simple first-order decay curve. However, when total BTEX is plotted versus time (Figure 3c), the lag and asymptotic periods disappear and the experimental results closely approximate a first-order decay process. The first-order decay rates for individual compounds reported in Table 2 are for the period of rapid biodegradation and are not representative of the entire incubation period. Use of these rates in a simple first-order decay function will substantially underestimate the time required for biodegradation.

Biodegradation in the end-plume RP microcosms was variable. Some microcosms exhibited clear evidence of biodegradation by day 106 while other microcosms did not show any evidence of biodegradation after 327 days (Figure 4). Toluene and *o*-xylene degradation appeared to proceed concurrently starting at 106 days (Figures 4b and 4c). On days 184 and 253, there was clear evidence for toluene and *o*-xylene degradation in all microcosms sampled. Yet on the last two sampling dates, the evidence was ambiguous as toluene had degraded in three of six microcosms sampled and *o*-xylene had degraded in only two of six. Benzene, *m*-xylene and ethylbenzene also degraded in selected microcosms but usually only when toluene and *o*-xylene were also depleted (Figure 4). Estimation of first-order decay rates was not appropriate at this location, since the calculated rate would be more a function of the random order of microcosm sampling than the actual rate of biodegradation. It was not possible to conclusively identify the electron acceptor in the end-plume microcosms because of the presence of high concentrations of dissolved sulfate and interferences in the solid-phase iron analyses.

Field (Borden *et al.*, 1995) and laboratory results indicate that at the RP site, the various BTEX components biodegrade in a sequential process: toluene and *o*-xylene are usually depleted first, followed by benzene and/or *m*-xylene and finally ethylbenzene. In the mid-plume microcosms, this pattern varied somewhat. The *m*-xylene biodegradation began without a lag but then slowed once toluene and *o*-xylene degradation began. At this location in the aquifer, toluene and *o*-xylene concentrations are low (~5 µg/Liter), but significant quantities of *m*-, *p*-xylene still remain (>1000 µg/Liter). These results suggest that *m*-xylene degradation was occurring *in-situ* at the time of sediment removal. However, toluene and *o*-xylene were preferentially degraded when elevated levels of these compounds were added to the microcosms. Benzene biodegradation did not begin until after 180 days. By this time, toluene and the xylene isomers were depleted to less than 10 µg/Liter. In the end-plume microcosms, the added compounds also degraded sequentially; however, toluene and *o*-xylene degradation did not occur until after 106 days (Figure 4). At this time, the various factors that control the order of biodegradation are not fully understood . Clearly, there are some interactions between the degradation of the different compounds. As discussed above, the presence of other organic substrates, including acetate, amino acids and propionate, has

TABLE 2. *First-order Decay Rates from the RP Site*

	Mid-Plume Microcosms		Mid-Plume In-Situ Columns		Field
Compound	Decay rate[a] (d^{-1})	Decay Period (days)	Decay rate[a] (d^{-1})	Decay Period (days)	Decay rate[b] (d^{-1})
Benzene	0.024	184-403	Test 1: 0.0049 Test 2: 0.023	Test 1: 155-251 Test 2: 41-181	0.0002
Toluene	0.045	22-120	NS[c]		0.0021
Ethylbenzene	NS[c]		Ns[c]		
o-Xylene		37-120	NS[c]		0.0021
m-, *p*-Xylene	0.020[d]	0-184	0.0143	121-251	0.0013
Total BTEX	0.0066	0-403	0.0029	121-251	0.0011

[a] *Rates represent the difference between the microcosms and control abiotic loss rate over the period of decay.*

[b] *Field decay rates were calculated over the entire length of the plume.*

[c] *Microcosms and control abiotic loss rates were not significantly different at the 99% level.*

[d] *Only m-xylene was present in the microcosms.*

been reported to inhibit anaerobic degradation of toluene and *o*-xylene. While only BTEX concentrations were quantified in the present work, the gas chromatograms from the mid-plume microcosms show that a large number of unidentified compounds were present at low concentrations at the start of the experiment. Over the course of the experiment, these compounds also disappeared.

The lag period prior to biodegradation varied significantly among sampling locations at the RP site and among individual microcosms. The exact cause of this variability is unknown but is believed to be due to differences in prior adaptation to BTEX, electron acceptor availability and aquifer geochemistry. In the source area, field monitoring data indicated that concurrent BTEX biodegradation and sulfate reduction were occurring immediately downgradient of the gasoline spill (Borden *et al.*, 1995). Consequently, sediment and ground water were collected from an area with significant concentrations of BTEX (26 mg/Liter) and sulfate (~35 mg/Liter) in the ground water. In retrospect, it appears that this was an area where active sulfate reduction was not occurring, since significant concentrations of sulfate were still present in the ground water. In the end-plume location, there was tremendous variability in aquifer geochemistry in the immediate area where the sediment was collected. Prior to construction of the microcosms, the sediment from each location was blended and passed through a No. 8 sieve to homogenize the sediment. The highly variable response in the destructively sampled microcosms from the end-plume location indicates that this procedure was not sufficient to eliminate differences among replicate microcosms.

In-Situ Column Results

Two sets of column experiments were performed at the mid-plume location at the RP site for comparison with the microcosm results. In each set of experiments, two microcosm columns were operated in parallel with one abiotic control. All microcosm and abiotic control columns exhibited an initial concentration decrease of several hundred μg/Liter because of sorption to the aquifer sediment (Beckman, 1994). The concentrations of hydrocarbons in the abiotic columns remained constant or declined slowly after the initial drop, indicating that biological activity or short-circuiting did not occur in the control columns. The DO concentration remained low (<0.2 μg/Liter) throughout all experiments. Biological loss rates were calculated as the difference between the rates in the microcosms and the abiotic controls, when these rates were statistically different. The microcosm decay rate was estimated by pooling the result from the two microcosm columns over the period of active biodegradation.

The first set of experiments was performed using ground water from a nearby well that was depleted in toluene and *o*-xylene but contained higher concentrations of benzene, ethylbenzene, *m*-, *p*-xylene, pseudocumene (1,2,4-trimethylbenzene) and mesitylene (1,3,5-trimethylbenzene). Samples were collected monthly for approximately 250 days when the experiment was terminated because of limited sample volume. The *m*-, *p*-xylene, benzene and pseudocumene biodegraded after initial lag periods that varied from 85 to 121 days. By day 251, *m*-, *p*-xylene had decreased by over 90% (Figure 5), benzene had decreased by 50% (Figure 6a) and pseudocumene had decreased by 75% to 90%. In the control column, *m*-, *p*-xylene had decreased by over 48%, benzene had decreased by 22% and pseudocumene had decreased by 35%. There was no evidence of toluene, *o*-xylene, ethyl-benzene or mesitylene biodegradation in either microcosm column. The absence of toluene and *o*-xylene biodegradation was likely due to the low initial concentration of these compounds (<50 μg/Liter). In the microcosm columns, DO remained below detection (<0.2 mg/Liter), sulfate declined slightly from 1.3 mg/Liter to the detection limit (~0.3 mg/Liter) and pH remained constant at 6.3. In the first microcosm column, dissolved iron increased from 111 to over 200 mg/Liter; while in the second microcosm column, dissolved iron increased from 99 to 140 mg/Liter.

In the second set of experiments, the columns were reloaded with ground water that contained higher concentrations of benzene (1,000 to 1,300 μg/Liter) and very low concentrations of TEX (~ 20 μg/Liter) to determine if benzene biodegradation would continue. Benzene biodegradation began after a 41-day lag period, and by day 334 benzene had declined from over

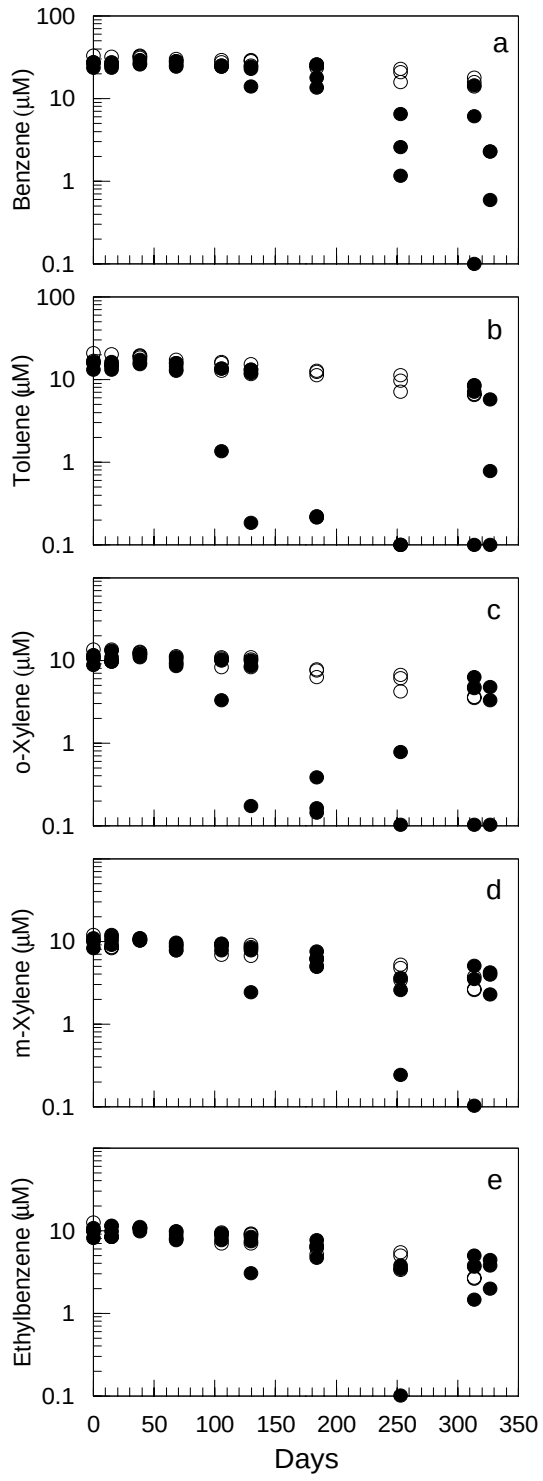

Figure 4. Variation in (a) benzene, (b) toluene, (c) o-xylene, (d) m-xylene and (e) ethyl-benzene in microcosms constructed with material from the RP end-plume location. Microcosm (•) and control (o) results are from individual destructively sampled microcosms. Three microcosms and three controls were sampled at each time point, except the last time point, when no control was sampled.

1,000 µg/Liter to 11 µg/Liter in column 1 and to 8 µg/Liter in column 2 (Figure 6b). The ground water used to reload the in-situ column was obtained from a nearby multilevel sampler. Previous monitoring had indicated that the sulfate concentration of this ground water was very low (~1 mg/Liter). However, after reloading the columns, sulfate concentrations in microcosm columns 1 and 2 were 85 and 65 mg/Liter, respectively. Additional monitoring confirmed that a pulse of high sulfate ground water had migrated past the multilevel sampler intake at the time ground water was collected for injection into the in-situ columns. Over the course of this experiment, sulfate remained constant in column 2 but declined by 25% in column 1, and dissolved iron remained constant in both columns. Data are insufficient to positively identify the electron acceptor used for benzene degradation in the in-situ column experiments.

First-order Biodegradation Rates

First-order biodegradation rates from the in-situ column experiments are compared to the biodegradation rates from the laboratory microcosms and field measurements in Table 2. Biodegradation rates for *m*-, *p*-xylene in the in-situ columns and *m*-xylene in the laboratory microcosms were similar. However, the lag period prior to the start of biodegradation was longer in the in-situ columns. The lag period prior to benzene biodegradation in the laboratory microcosms and the first in-situ column experiment was similar. However, the rate of benzene biodegradation in the first in-situ column experiment was a factor of five lower than the laboratory microcosm rate. During this experiment, benzene biodegraded concurrently with *m*-, *p*-xylene. However, in the microcosms, *m*-xylene was completely degraded before the start of benzene biodegradation. In the second in-situ experiment, only benzene was present and the benzene biodegradation rate was similar to the laboratory microcosms.

In most cases, the measured biodegradation rates for individual compounds are comparable in both columns and microcosms but are one or two orders of magnitude higher than rates estimated from field investigations (Borden *et al.*, 1994). One likely cause of this difference is the procedure used to calculate biodegradation rates. In the laboratory microcosms and the in-situ columns, there is a definite lag period prior to the start of biodegradation. The laboratory and in-situ column rates were calculated during the period of active biodegradation after the lag

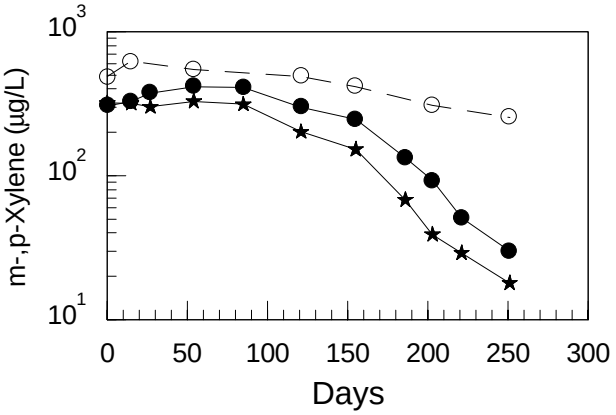

Figure 5. Variation in *m*-, *p*-xylene in the first set of in-situ test columns. Open symbols are abiotic controls; solid symbols are microcosm columns.

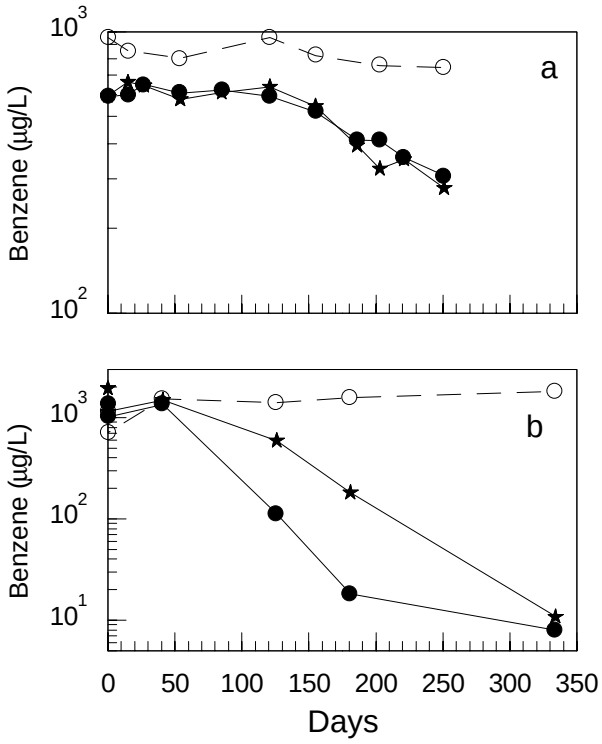

Figure 6. Variation in benzene in in-situ columns: (a) initial experiment; and (b) after reloading with benzene only. Open symbols are abiotic controls; solid symbols are microcosm columns.

period had ended. In the field, it is usually not possible to identify the zones where biodegradation is most active, and the reported degradation rates are for the travel time over the entire plume.

The large differences between laboratory microcosm, in-situ column and field-scale biodegradation rates for individual compounds could be reduced by using a grouped parameter such as total BTEX. In the laboratory microcosms, one or more BTEX components were degrading throughout the experiment, so a simple first-order decay model closely matched the total BTEX results for the entire experiment with no observable lag in total BTEX biodegradation (Figure 3c). In the field, one or more BTEX components are biodegrading at any location, and consequently a first-order decay function more closely matches the field data throughout the entire length of the plume. When laboratory microcosm, in-situ column and field rates are compared, biodegradation rates for total BTEX are much more consistent than the rates for individual compounds. For example, the laboratory biodegradation rate for benzene was 120 times the field rate, while the laboratory rate for total BTEX was only 6 times the field rate. The highest benzene degradation rate in the in-situ columns was 115 times the field rate, while the total BTEX degradation rate in the columns was only 2.6 times the field rate.

A second potential cause for the observed differences between laboratory microcosm, in-situ column and field biodegradation rates is spatial variations in biological activity. The rates reported in Table 2 were calculated from column and laboratory measurements at a single location (mid-plume). At a second location (source area) there was no evidence of BTEX biodegradation, and in a third location (end-plume) the results were variable. In contrast, the field degradation rates were estimated from monitoring well data collected along the length of the plume and should represent the large scale, spatially-averaged rate.

Conclusions and Recommendations

Anaerobic Biodegradation of BTEX

1. Benzene, toluene, ethylbenzene and the xylene isomers are anaerobically biodegradable under ambient subsurface conditions using ferric iron, sulfate and/or carbon dioxide as terminal electron acceptors.
2. A distinct order of biodegradation is often observed, with toluene being the most rapidly biodegraded compound. However, this order may vary from site to site. For example, at the Rocky Point site, *o*-xylene was rapidly biodegraded; while at the Sleeping Bear site, no significant biodegradation of *o*-xylene was observed.
3. The more easily biodegradable compounds (toluene, *o*-xylene, *m*-xylene) appear to anaerobically biodegrade to a low but detectable concentration (10 to 30 µg/Liter) after which biodegradation slows or stops. It is not clear whether biodegradation of these compounds will continue once the more difficult to degrade compounds are depleted.
4. Use of a simple first-order decay model does not adequately describe the anaerobic biodegradation of individual compounds in laboratory microcosms or in-situ columns. To accurately simulate the anaerobic biodegradation of individual compounds, a model that includes two variables will be required: (1) the lag period prior to biodegradation; and (2) the rate of biodegradation.
5. The lag period prior to the start of anaerobic BTEX biodegradation varies from compound to compound and from site to site. Until the source of this variability is better understood, it will not be possible to use laboratory microcosms or in-situ columns to accurately predict the time required for the field biodegradation of individual compounds.
6. Destructive microcosms yield only one concentration measurement for each independent experiment (microcosm) and are poorly suited to generating the data required to fit a two-parameter model. Consequently, use of destructive microcosms, as specified in the EPA protocol for estimation of anaerobic microbiological transformation rate data, is not appropriate when the lag period prior to the start of biodegradation is significant.
7. Anaerobic biodegradation of total BTEX more closely approximates a first-order decay curve than the biodegradation of the individual compounds. First-order decay rates for total BTEX estimated from laboratory microcosms, in-situ columns and field monitoring data also appear to be more consistent than the rates for individual BTEX components.

Use of Laboratory Microcosms and In-situ Columns to Evaluate Natural Attenuation of BTEX

1. At this time, biodegradation rates for individual compounds derived from laboratory microcosms and in-situ columns cannot be reliably used to estimate the time required for complete biodegradation in the field.

2. Laboratory microcosms are useful for: (1) demonstrating that a compound of regulatory concern can and does biodegrade under ambient subsurface conditions; and (2) evaluating the effect of different environmental variables on the rate and extent of biodegradation.
3. In-situ columns may also be used to evaluate compound biodegradation under ambient conditions and the effect of different amendments. In-situ columns are relatively simple to install and operate, closely replicate ambient conditions, and result in minimal disturbance of the aquifer material.
4. Total BTEX may be a more appropriate parameter for describing natural attenuation than the concentration of individual compounds. Biodegradation of total BTEX more closely matches a first-order decay curve than the individual compounds. The degradation rate for total BTEX also appears to be more consistent than biodegradation rates for individual compounds. However in some cases, it may be necessary to model individual compounds to accurately assess the risk to human health and the environment.
5. Estimation of field biodegradation rates from limited point measurements (laboratory microcosms or in-situ columns) will be difficult because of spatial variations in biological activity.

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Quality Assurance Statement

All research projects making conclusions or recommendations based on environmentally related measurements and funded by the Environmental Protection Agency are required to participate in the Agency Quality Assurance Program. This project was conducted under an approved Quality Assurance Program Plan. The procedures specified in the plan were used without exception. Information on the plan and documentation of the quality assurance activities and results are available from the Principal Investigator.

References

Becker, M. T. 1992. Iron Reduction in a Gasoline-Contaminated Aquifer Containing Glauconite. M.S. Thesis, North Carolina State University, Raleigh, North Carolina. 97 pages.

Beckman, M. 1994. In-Situ Measurement of Intrinsic Bioremediation Rates, M.S. Thesis, North Carolina State University, Raleigh, North Carolina. 121 pages.

Borden. R. C., C. A. Gomez, and M. T. Becker. 1994. Natural Bioremediation of a Gasoline Spill, pp. 290-295. *In* R. E. Hinchee, B. C. Alleman, R. E. Hoeppel, and R. N. Miller (ed.). *Hydrocarbon Bioremediation*, CRC Press, Boca Raton, Florida.

Borden. R. C., C. A. Gomez, and M. T. Becker. 1995. Geochemical Indicators of Natural Bioremediation. Ground Water **33:**180-189.

Dunlap, W. J., J. F. McNabb, M. R. Scalf, and R. L. Cosby. 1984. Sampling for Organic Chemicals and Microorganisms in the Subsurface. EPA/600/2-77/176, U.S. Environmental Protection Agency, Ada, Oklahoma.

Edwards, E. A., and D. Grbić-Galić. 1994. Anaerobic Degradation of Toluene and *o*-Xylene by a Methanogenic Consortium. Appl. Environ. Microbiol. **60:**313-322.

Gillham, R. W., R. C. Starr, and D. J. Miller. 1990. A Device for In-situ Determination of Geochemical Transport Parameters; 2 Biochemical Reactions. Ground Water **82**: 858-862.

Gomez, C. A. 1993. Characterization of a Dissolved Hydrocarbon Plume, M.S. Thesis, North Carolina State University, Raleigh, North Carolina. 107 pages.

Hunt, M. J., M. B. Shafer , M. A. Barlaz, and R. C. Borden. 1997. Anaerobic Biodegradation of Alkylbenzenes in Laboratory Microcosms Representing Ambient Conditions. Bioremediation Journal **1**(1):53-64.

West, C. C., W. G. Lyon, D. L. Ross, and L. K. Pennington. 1994. Investigation of the Vertical Distribution and Morphology of Indigenous Organic Matter at the Sleeping Bear Site, Michigan. Environ. Geol. **24**:176-187.

Wilson, J.T., D. H. Kampbell, and J. Armstrong. 1994. Natural Bioreclamation of Alkylbenzenes (BTEX) from a Gasoline Spill in Methanogenic Groundwater, pp. 201-218. *In* R. E. Hinchee, B. C. Alleman, R. E. Hoeppel, R. N. Miller (ed.), *Hydrocarbon Bioremediation*. CRC Press, Inc., Boca Raton, Florida.