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In Situ BTEX Biotransformation under Enhanced Nitrate- and Sulfate-Reducing Conditions

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In situ anaerobic biotransformation of BTEX (benzene, toluene, ethylbenzene, *o*-xylene, and *m*-xylene) was investigated under enhanced nitrate- and sulfate-reducing conditions. Controlled amounts of BTEX compounds added to slugs of treated groundwater were released into a gasoline-contaminated aquifer at Seal Beach, CA. In a series of studies, the slugs, 470–1700 L in volume, were released into the aquifer through a multi-port injection/extraction well and were subsequently withdrawn over a 2–3-month period. To evaluate unamended in situ conditions, the injectate was treated with granular activated carbon (GAC) and augmented with bromide as a tracer. To evaluate nitrate- and sulfate-reducing conditions, the injectate was also deionized and augmented with 200–300 μg/L BTEX, nitrate or sulfate, and background electrolytes. Under unamended conditions, transformation appeared to be limited to the slow removal of toluene and *m,p*-xylene (i.e., sum of *m*+*p*-xylene). Under nitrate-reducing conditions, toluene, ethylbenzene, and *m*-xylene were transformed without a lag phase in less than 10 days, and *o*-xylene was transformed in 72 days. Under sulfate-reducing conditions, toluene, *m*-xylene and *o*-xylene were completely transformed in less than 50 days, and ethylbenzene was removed in 60 days. Benzene appeared to be removed under sulfate-reducing conditions, but the trend was pronounced only at some levels. A two-dimensional model is presented for the evaluation of reactive solute behavior in such slug tests. For compounds that are transformed without a lag phase, zero-order kinetics appears to be more applicable than first-order kinetics.

Introduction

Leaks are estimated to occur at approximately one-third of all underground motor fuel storage facilities (*1*) causing a significant groundwater contamination problem. Some of the contaminants of greatest concern are benzene, toluene, ethylbenzene, and the xylene isomers (BTEX), which are relatively mobile as well as toxic and/or confirmed carcinogens (*2*). BTEX and other hydrocarbon compounds are rapidly degraded by aerobic bacteria (*3–5*). However, aerobic processes are limited by the slow rate at which oxygen can be supplied to the contaminated zone and, therefore, are significant only at the fringes of plumes (*6, 7*). Anaerobic processes that occur inside the plumes are not well understood, although they may contribute significantly to intrinsic (or natural) hydrocarbon mineralization.

Anaerobic processes utilizing alternate electron acceptors are increasingly being considered as an attractive option for bioremediation of hydrocarbon-contaminated sites. Possible alternate electron acceptors include nitrate (*8–18*), iron(III) (*19, 20*), and sulfate (*13, 21–26*). Several authors also have shown mineralization of toluene as well as other alkylbenzenes in fermentative/methanogenic enrichments (*27–29*).

It is currently not possible to predict anaerobic BTEX transformation because the factors that promote or inhibit the process are not well understood. Furthermore, it is unknown whether rates measured in the laboratory can be applied to the field. Laboratory studies have shown that degradation rates can be sensitive to the presence of readily degradable co-substrates and geochemical factors. For instance, when multiple BTEX compounds are present simultaneously, anaerobic biotransformation was found to be sequential with toluene being the most readily degraded compound followed by *p*- and *o*-xylene (*13, 21, 22*). The place of ethylbenzene in the sequence depends on the geochemical conditions: it is high under nitrate-reducing conditions but low under sulfate-reducing conditions. Benzene is generally the most persistent compound, although it has been shown to be degraded under methanogenic (*28*), sulfate-reducing (*24, 30*), and iron-reducing conditions (*31*). Hydrogen sulfide inhibits degradation of BTEX compounds under sulfate-reducing conditions (*22, 23, 32*). Ferric or ferrous iron may aid in initiating or accelerating BTEX transformation by removing free hydrogen sulfide from solution, thereby preventing sulfide toxicity (*23, 32*). Field studies have demonstrated that nitrate can enhance BTEX transformation at hydrocarbon-contaminated sites (*33–35*). Thierrin et al. observed toluene, *p*-xylene, and naphthalene transformation in a sulfate-reducing gasoline-contaminated aquifer (*36*).

This paper presents field evidence for the biological removal of BTEX compounds under enhanced nitrate- or sulfate-reducing conditions. The methodology used in this field study involved slug (batch) tests in which a test zone of an aquifer was incubated with groundwater of a known composition. To control geochemical conditions, the slugs of groundwater (the injectate) were first treated to remove potentially interfering solutes including BTEX and other organic compounds, oxygen, and other electron acceptors. Prior to injection, the injectate was augmented with benzene, toluene, *o*- and *m*-xylene, ethylbenzene, nitrate or sulfate, tracer, and electrolytes as needed for the test.

Experimental Design

Site Description. Tests were conducted at a gasoline site located on the premises of the Seal Beach Naval Weapons Station in southern California. The hydrogeology, the extent of subsoil contamination by weathered gasoline (*37*), and the water chemistry (*38*) were described previously. The site is located approximately 1 km inland from the Pacific Ocean near a marsh and wildlife refuge. The gasoline leak was discovered in 1984. Approximately 20 000 L of unleaded gasoline leaked into the unconfined aquifer. The water table is approximately 2 m below the surface, and the aquifer consists of sandy/silty alluvial and coastal deposits. Schroeder suggested that the plume was of radial shape consistent with the low regional hydraulic gradient (*37*). Spreading of the plume was presumed to be caused by small fluctuations (up to 4 cm) of the water table induced by the tide.

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The redox conditions at the site varied and were more reducing toward the center of the plume. In uncontaminated wells, nitrate and sulfate concentrations were typically 15 and 100 mg/L, respectively. In wells near the contamination source, methane was detected, nitrate was absent, and sulfate was significantly depressed, consistent with methanogenic, denitrifying, and sulfidogenic conditions (data not shown). In the area of the test zone (designated as EO), initial conditions were reducing with redox potentials below −100 mV and nitrate and sulfate concentrations approximately 5 and 85 mg/L. BTEX concentrations were highest close to the original tank location and rapidly dropped off toward the fringes of the plume. Initially the water pumped from the test zone contained on average 750 μg/L benzene, 74 μg/L toluene, 89 μg/L ethylbenzene, 16 μg/L *m*- and *p*-xylene and 150 μg/L *o*-xylene. *m*- and *p*-xylene were not resolved chromatographically and are reported as *m,p*-xylene. The background concentration of bromide was approximately 0.6 mg/L.

Aquifer material consisted of quartz sand with less than 3% $CaCO_3$, low (<2%) intraparticle porosity, and low organic carbon (0.02%) (C. Schueth, personal communication) and exhibited only a weak sorptive capacity for BTEX compounds (K_d for toluene 0.17 L/kg). The BTEX biotransformation potential of contaminated aquifer material was reported previously (*13, 22, 29*). The sediments were also shown to exhibit a significant nitrate and sulfate demand presumably due to uncharacterized organics.

Slug Tests. The slug test methodology used involved incubating an unconfined test zone with 470−1700 L of groundwater. A schematic top view and cross section of the test zone indicating the sampling device are shown in Figure 1. Slugs of groundwater were injected into the unconfined test zone through a multi-level test well designated as EO. For simplicity, the test zone is assumed to be cylindrical with a radius *r*. After incubation varying from days to months, samples were withdrawn periodically, thereby reducing the size of the test zone. Samples were routinely analyzed for tracer (bromide), BTEX, the electron acceptors nitrate and sulfate, and reduced forms of the electron acceptors (nitrite and hydrogen sulfide).

A sequence of seven slug tests, designated as EO1−EO7, were conducted. The injectate was treated as needed to create specified geochemical conditions (Table 1). The contaminant mass present during EO1−EO3 originated from the spill and was unknown, making it difficult to quantitatively evaluate BTEX removal. After EO3, BTEX compounds in the test zone were depleted and, consequently, amended to the slugs. In all subsequent experiments, BTEX mass removal could be evaluated by subtracting the mass recovered from the mass injected. EO1 served as a tracer test in which the injectate was groundwater treated by GAC and amended with bromide salt. The low levels of nitrate (4−5 mg/L) present in the extracted groundwater were removed (presumably by denitrification) during storage of the extracted groundwater in the tank. In experiment EO2, the injectate was deionized to remove nitrate and sulfate, to limit BTEX removal by denitrifying and sulfate-reducing bacteria, and to observe the capacity of the sediments to desorb BTEX. The injectate contained only the tracer, background electrolytes, and no added electron acceptor. In EO3, the test zone was inundated with nitrate to stimulate BTEX removal. The injectate in EO3 was amended with tracer, 209 mg/L nitrate, and electrolytes. For reasons that are not explained, EO3 produced an anomalous response in that the tracer, nitrate, and BTEX compounds decreased nearly proportionally during the experiment. Perhaps N_2 formed by denitrification exceeded its aqueous solubility (approximately 20 mg/L) and filled a fraction of the pore volume, leading to clogging [mineralization of 17 mg/L hydrocarbon compounds (as toluene) with 81 mg/L nitrate may form 20 mg/L N_2].

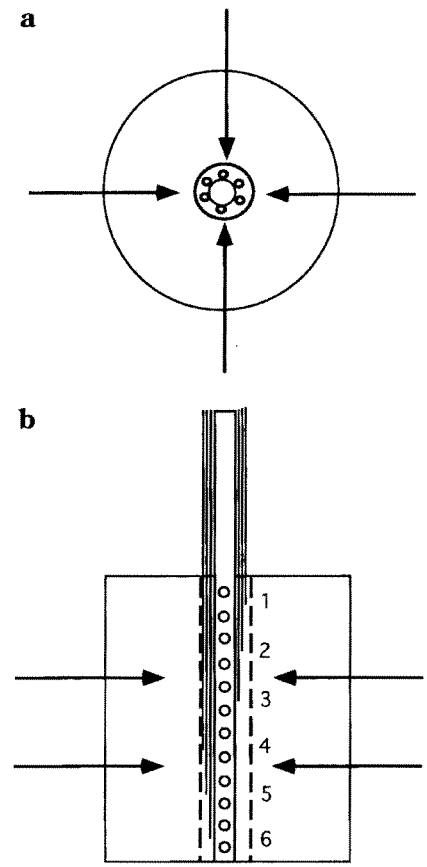

FIGURE 1. Schematic of test zone and sampling device inside the well casing. Arrows indicate flow during well purging and sampling. (a) view of converging flow (in the absence of significant regional groundwater flow). (b) Cross section of test zone. Circles in well indicate level of sampling tubes. The circles indicate the depths of the individual sampling tubes.

At the end of EO3, all BTEX compounds were close to or below their detection limit, indicating that the sediments no longer served as a BTEX source. Consequently, in all subsequent tests, BTEX compounds (except p-xylene) were added to the slugs at concentrations between 200 and 300 μg/L, as indicated in Table 1. Experiments EO4 and EO5 were designed to evaluate BTEX removal under nitrate-reducing conditions. Similarly, experiments EO6 and EO7 were designed to evaluate BTEX removal under sulfate-reducing conditions. Anticipating utilization of the electron acceptors by organic compounds other than the amended BTEX, the electron acceptors were added at concentrations above the theoretical demand exerted by the amended BTEX compounds. In EO7, characteristic byproducts of anaerobic alkylbenzene metabolism including benzylsuccinic acid were analyzed along with the regular suite of analytes to confirm the anaerobic nature of the transformation (*39*).

Wells were installed in December 1992. A multilevel observation well designated as east observation (EO) was used for the slug tests. A well EI located 1.65 m downgradient from the EO well was used to observe contaminant migration away from the test zone due to regional flow. The wells were instrumented with samplers that provided both composite and depth-specific samples to be collected as shown schematically in Figure 1. The test zone was approximately 1.5 m deep starting from approximately 0.3 m below the water table. The samplers were made of bundles of stainless steel tubing and consisted of a central 1/4-in. composite tube and six individual 1/8-in. tubes. The composite tube had 12 1.56-

TABLE 1. Summary of Experimental Parameters

experiment	EO1	EO2	EO3	EO4	EO5	EO6	EO7
pretreatment[a]	GAC/P	GAC/DI/P	GAC/DI/P	GAC/DI/P	GAC/DI/P	GAC/DI/P	GAC/DI/P
amendments	Br^-	Br^- + EI[b]	Br^- + EI + NO_3^-	Br^- + EI + NO_3^-	Br^- + EI + NO_3^-	Br^- + EI + SO_4^{2-}	Br^- + EI + SO_4^{2-}
BTEX source	sediments[c]	sediments	sediments	injectate	injectate	injectate	injectate
vol injected (L)	535	490	1700	901	995	750	837
total sample vol extracted (L)	1626	1588	3077	525	525	479	490
buffer vol (L)				948	853	735	932
test duration (days)	60	97	134	79	67	60	80
bromide (mg/L)	60	34	67	67.2	44.6	56.4	48
nitrate (mg/L)	<1	<1	209	226.8	37.2	0	0
sulfate (mg/L)	97	9	9	2.7	2.0	44.6	15.9
benzene (μg/L)				241	255	204	250
toluene (μg/L)				286	286	259	282
ethylbenzene (μg/L)				286	293	296	298
m-xylene (μg/L)				211	236	227	247
o-xylene (μg/L)				250	0	277	294

[a] GAC, granulated activated carbon; DI, mixed-bed deionization; P, helium purging. [b] Electrolytes. [c] BTEX desorbing from sediments after reinjection of purified water.

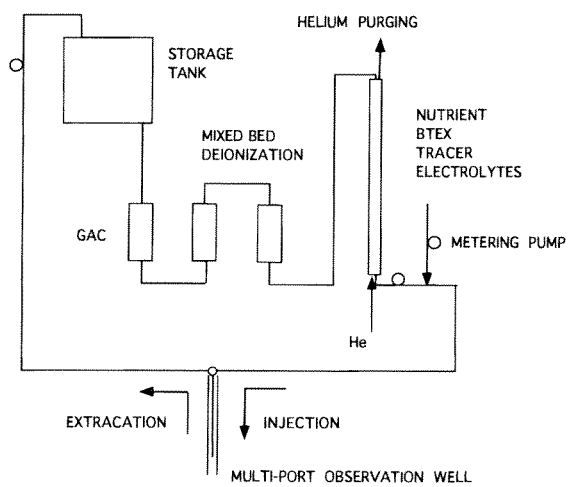

FIGURE 2. Schematic of groundwater pretreatment processes.

mm holes drilled into it starting 6.25 cm from the bottom and were spaced 12.7 cm apart. The lower end was capped. The individual sample ports were spaced 25.4 cm apart starting 12.7 cm from the bottom of the composite tube. The six multilevel sample ports had filters on the inlet, which consisted of glass wool surrounding the tip of the tube that were then held in place by nylon fabric. After placing the samplers in the 2-in. well, 3-mm glass beads were added to the static groundwater level in order to reduce the void volume in the well casing. Samples collected from these multilevel samplers were numbered from 1 (at the top) to 6 (at the bottom).

Groundwater pretreatment and amendments for the experiments are indicated in Table 1. A schematic of the groundwater pretreatment process is indicated in Figure 2. Groundwater was prepared by pumping water from the EO well into a tank where it was stored for several hours until use. From the tank, the water was pumped through two GAC filters providing approximately 2 min contact time to remove BTEX and other organic compounds. The GAC effluent was filtered with a 1-μm cotton filter. The existing electron acceptors were removed by deionization using multiple mixed-bed ion exchange resin canisters. The effluent of the ion exchange bed was monitored with a 200 kΩ conductivity detector light. To remove dissolved oxygen and residual BTEX (to <1 μg/L), the deionized water was passed through a helium-purged gas stripping tower. The dissolved oxygen (DO) concentrations in the stripping tower effluent were measured with a DO probe and were below 0.15 mg/L (with few exceptions) and were assumed to be rapidly quenched near the well either by aerobic bacteria or reduced chemical species. The stripping tower was composed of a 3-m-long 3-in. PVC pipe and was filled with 1.6-cm polypropylene Flexrings (Koch). The slugs were metered into the well through the composite tube at an average flow rate of 1.5 L/min to minimize pressure mounding during the injection. The deionized water was amended with the chloride salts of calcium, magnesium, and sodium such that the resulting electrolyte solution was approximately equal in ionic strength to the groundwater. Sodium bromide was added as a conservative tracer, and nitrate and sulfate were added as electron acceptors as required.

In EO1–EO3, the tracer concentration began to decrease after extracting approximately 40% of the test slug volume. At this point, the slug water began to mix with groundwater that was infiltrating into the test zone, thus gradually changing the water composition. In EO4–EO7, the test zone was surrounded by a "geochemical" buffer of the same composition as the test slug except that the organics and tracer were not added. This geochemical buffer extended the period during which the chemical composition of the aqueous matrix was constant and contaminant transformation could be observed under constant conditions. The geochemical buffer was injected prior to the actual test slug and was approximately of equal volume as the test slug volume (Table 1). In EO6 and EO7, the buffers were embedded in an outer nitrate buffer (surrounding the sulfate slug) to assure biological removal of BTEX compounds that might not be recovered.

Sampling and Analysis. Samples were collected weekly or biweekly during experiments that lasted for 2–4 months. After purging the well of approximately 50 L (approximately 2 L through each of the individual sampling lines and the balance through the composite), 40-mL samples were taken from each individual sampling port and from the composite tube using a sampling manifold. Bottles were filled slowly leaving no headspace. The duplicate BTEX samples were preserved with 1 drop of concentrated HCl. BTEX compounds were analyzed using a purge-and-trap sampler connected to a gas chromatograph with a photoionization detector. The GC procedure used did not separate *m*-xylene and *p*-xylene. During EO1–EO3, when the contaminated sediment was the source of the BTEX, the two compounds could not be studied individually. *p*-Xylene was not included in EO4–EO7. The anions Br^-, NO_3^-, and SO_4^{2-} were analyzed by ion chromatography as described elsewhere (*40*).

The initial concentrations (denoted as C_0) were determined immediately after injection by collecting samples taken from

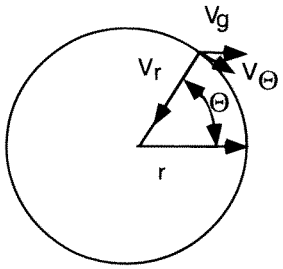

FIGURE 3. Flow components during steady-state pumping: V_r, radial flow; V_g, regional groundwater flow; V_Θ, radial flow in Θ direction.

inside the well casing, i.e., without flushing the borehole. Thereafter, the samples were collected at regular intervals with sufficient flushing of the well borehole to produce water from outside of the well casing. Flushing required a minimum of 50 L but was increased in some cases to accelerate extraction of the bromide pulse. In EO1, 60 L was extracted per sample session for the first pore volume, then 140 L per sample session for the next 1.5 pore volumes, and 260 L in the final sample session. Tests EO2 and EO3 followed similar procedures. EO4–EO7 were sampled 9–12 times before the remaining slug was extracted by continuous pumping.

Model Development

Governing Equations. The model assumes that a fully penetrating well is imbedded in an infinite homogeneous confined aquifer and that the flow velocity and concentration are depth-averaged, resulting in a two-dimensional flow and transport representation. In the absence of natural groundwater flow and dispersion, the flow is radial and the injected slug forms a concentric cylinder as shown in Figure 3. Release of the slugs lasted 5–15 h (at 1.5 L/min), and the effect of the natural gradient flow was insignificant during injection. During the 2–3-months extraction phase, however, the natural gradient flow was significant and required consideration of a two-dimensional model in the x–y plane.

Using the coordinate system shown in Figure 3, for constant pumping rate and groundwater flow rate, the steady-state flow velocities in radial coordinates can be written as

$$v_r = -\frac{Q}{2\pi h\phi r} + v_g \cos\theta \tag{1}$$

$$v_\theta = -v_g \sin\theta \tag{2}$$

where v_r and v_θ are the velocity components in the r and θ directions, respectively, Q is the pumping rate, h is the aquifer thickness (in our case, h is taken to be equal to the length of the screened section of the well), ϕ is the aquifer porosity, and r is a radial coordinate measured from the center of the well. Diverging and counter-clockwise are defined as positive directions for the radial and angular coordinates, respectively. The transport of a reactive compound injected into the aquifer, undergoing zero-order biological transformation, is governed by the mass balance equation (*41*):

$$\frac{\partial C}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(rD_{rr}\frac{\partial C}{\partial r} + D_{r\theta}\frac{\partial C}{\partial \theta}\right) + \frac{1}{r}\frac{\partial}{\partial \theta}\left(\frac{1}{r}D_{\theta\theta}\frac{\partial C}{\partial \theta} + D_{r\theta}\frac{\partial C}{\partial r}\right) - \frac{\partial(v_r C)}{\partial r} - \frac{v_r C}{\partial r} - \frac{1}{r}\frac{\partial(v_\theta C)}{\partial \theta} - k_b \tag{3}$$

where C is the aqueous concentration of the compound, v_r and v_θ are velocities given by eqs 1 and 2, k_b is a zero-order reaction rate constant, and D_{rr}, $D_{\theta\theta}$, and $D_{r\theta}$ are the hydrodynamic dispersion coefficients, which are calculated using transformation rules from the longitudinal and transverse dispersion coefficients.

The hydrodynamic dispersion coefficient is normally written as a sum of the mechanical dispersion coefficient and molecular diffusion coefficient (*42*). Sufficiently close to the well, the contribution of molecular diffusion to radial dispersion can be neglected (*43*). Thus, the hydrodynamic dispersion coefficients may be approximated as

$$D_L = \alpha_L |v| \tag{4}$$

$$D_T = \alpha_T |v| \tag{5}$$

where α_L and α_T are the longitudinal and transverse dispersivity of the aquifer with $\alpha_L \gg \alpha_T$. The magnitude of the velocity is given by

$$|v| = \sqrt{v_r^2 + v_\theta^2} \tag{6}$$

The angle from the radial direction to the total flow direction at any given point can be calculated from the two velocity components as follows:

$$\beta = \arctan\left(\frac{v_\theta}{v_r}\right) \tag{7}$$

The desired dispersion coefficients can then be calculated using the following equations:

$$D_{rr} = \frac{D_L + D_T}{2} + \frac{D_L - D_T}{2}\cos(2\beta) \tag{8}$$

$$D_{\theta\theta} = \frac{D_L + D_T}{2} - \frac{D_L - D_T}{2}\cos(2\beta) \tag{9}$$

$$D_{r\theta} = \frac{D_L - D_T}{2}\sin(2\beta) \tag{10}$$

In the absence of the natural gradient, the corresponding transport equation is simplified into

$$\frac{\partial C}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(rD_{rr}\frac{\partial C}{\partial r}\right) - v_r\frac{\partial C}{\partial r} - k_b \tag{11}$$

with

$$v_r = -\frac{Q}{2\pi h\phi r} \tag{12}$$

Substitution of eq 12 into eq 11 yields the following:

$$\frac{\partial C}{\partial t} = \left(\frac{|A|\alpha_L}{r}\frac{\partial^2 C}{\partial r^2}\right) - \frac{A}{r}\frac{\partial C}{\partial r} - k_b \tag{13}$$

where $A = Q/(2\pi h\phi)$.

When $k_b = 0$, eqs 3 and 13 apply to the transport of a nonreactive tracer. It is noteworthy that the retardation factor of the substrate does not need to be considered since the injection volume is equal to the extraction volume. Thus, during extraction, solutes that sorb rapidly and reversibly produce the same response at the injection/extraction well as the tracer since the flow is reversed. These assumptions are consistent with the results of the preliminary sorption studies.

Initial and Boundary Conditions. The following initial and boundary conditions for the domain $\{r_w \le r \le r^*, 0 \le \theta \le \pi\}$ were used during extraction:

initial conditions

$$C(r,\theta,t)|_{t=0} = C_0, \text{ within plug (0 otherwise)} \tag{14}$$

boundary conditions

$$\left.\frac{\partial C(r,\theta,t)}{\partial r}\right|_{r=r_w} = 0 \quad (15)$$

$$C(r^*,\theta,t) = 0 \quad (16)$$

$$\left.\frac{\partial C(r,\theta,t)}{\partial \theta}\right|_{\theta=0} = 0 \quad (17)$$

$$\left.\frac{\partial C(r,\theta,t)}{\partial \theta}\right|_{\theta=\pi} = 0 \quad (18)$$

where C_0 is the concentration of the solute at the end of injection, r_w is the radius of the well, and r^* is the radius sufficiently far away from the plug flow radius at the end of the injected phase. Equation 14 states that before extraction started, the concentration of the injected compound is constant at C_0 within the plug volume and 0 outside of the plug volume. Equation 15 states that the mass flux into the well is solely due to advection, and eq 16 states that the concentration far away from the test zone is constant at all times at 0. Since the system in consideration is symmetric with respect to the direction of natural gradient flow, boundary conditions indicated in eqs 17 and 18 are used.

The above equations were solved numerically using the finite difference method. The presence of variable coefficients in the dispersion and advection terms required small spatial steps and, consequently, very small time steps.

Results and Discussion

Vertical Variability of Tracer Responses. Figure 4 shows the tracer responses of EO1 in the composite and the six multilevel sampling tubes. The bromide concentration increased from background to 60 mg/L and remained constant for the first four samples collected from all depths except level 6. At all levels except 6, mixing with groundwater became apparent when V/V_0 was approximately 0.4, indicated by the rapidly declining tracer concentrations. The rapid drop was followed by a long tail with concentrations approaching background levels by $V/V_0 = 3$. As is evident from Figure 4, the composite tracer response could be considered as a representative average for the tracer behavior as a whole. After extracting 1620 L ($V/V_0 = 3.0$), 96% of the bromide was recovered based on composite concentrations. At this point, the bromide concentrations were approximately 1.2 mg/L, 0.6 mg/L above the background concentration. The tracer concentrations measured in EO2 closely followed those obtained in EO1 (data not shown). The mass of bromide recovered in EO2 after $V/V_0 = 3$ was 93%.

Modeling the Response of Tracer and Reactive Solutes. An initial estimate for the longitudinal dispersivity was obtained assuming no groundwater flow using the method of Pickens and Grisak (*44*). Using the composite data of EO1 and assuming a porosity of 0.38, α was estimated as 3.5 cm. However, the early decrease and the long tail in tracer concentration could not be fitted using α as the sole fitting parameter. To account for the long tail and the discontinuities in the tracer response, it was necessary to account for the local groundwater flow and the actual pumping schedule. Molecular diffusion on the order of 1.0×10^{-5} cm^2/s was found to be insignificant. In Figure 5, the tracer data are compared with model predictions using longitudinal dispersivities of 3.0, 5.0, and 7.0 cm. The transverse dispersivities were assumed to be 1/50th of that for longitudinal dispersion. The model is relatively insensitive to this ratio within the range of reported values (*44*). To obtain the best fit for a given α, the groundwater velocity was adjusted. The best visual fit was obtained with $V_g = 0.7$ cm/day (which appears to be consistent with V_g values estimated from regional data) and $\alpha_L = 5.0$ cm. Discontinuities in the tracer response are

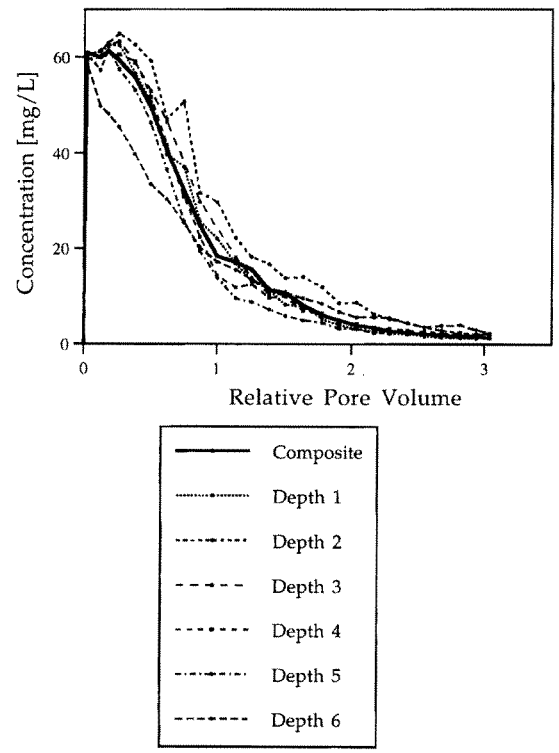

FIGURE 4. Depth-specific and composite tracer response of EO1.

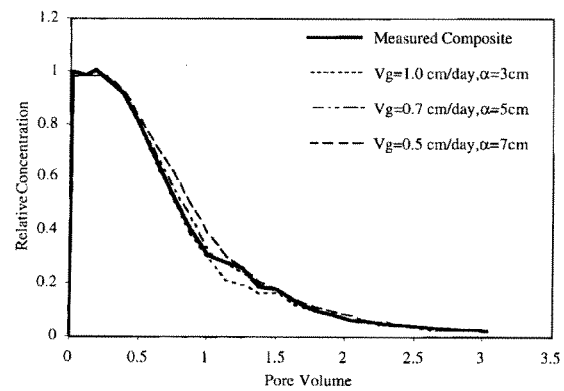

FIGURE 5. Composite tracer concentration of EO1 and model fit using a 2D-Model and the dispersivity and groundwater flow velocity as fitting parameters.

only noticeable between $V/V_0 = 0.8$ and 1.4. They become more pronounced as the groundwater velocity increases. At V_g significantly above 1 cm/day, the fraction of tracer that is recovered decreases. Fitting EO2 data yielded consistent estimates for v and α.

The predicted response of reactive solutes reacting with four different zero-order rate constants (k_b), 0, 0.01, 0.1, and 1.1 mg m^{-3} h^{-1}, are indicated in Figure 6. To account for dilution, the relative concentrations (C/C_0), were divided by the dilution factor indicated by the relative bromide concentration (Br/Br_0). The transformation rates were matched approximately to fit the rates observed in tests EO4–EO7 (discussed below). The rate curve remains constant at 1.0 for nonreactive solutes ($k_b = 0$), and the curves are linear for compounds that completely transform during extraction of undiluted injectate. For compounds that transform more slowly, the slope is constant during extraction of the undiluted slug, which ends approximately at day 30. When dilution

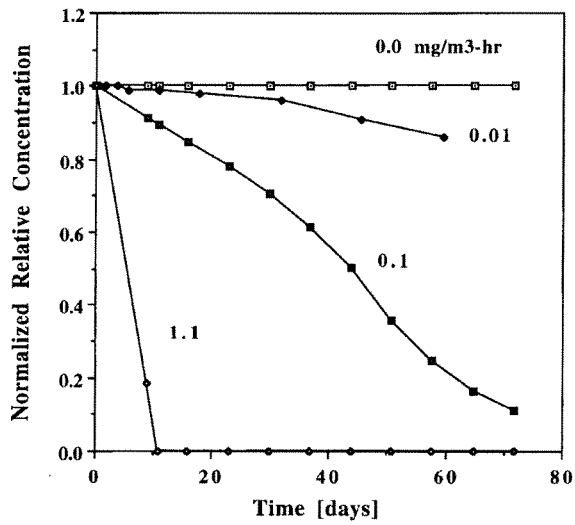

FIGURE 6. Predicted response for reactive solutes assuming different zero-order transformation rates.

begins, the curve bends downward, deviating from the linear decrease.

BTEX Behavior under Unamended Groundwater Conditions. The data of EO1 were used to evaluate BTEX removal under unamended (presumably sulfate-reducing) conditions, which may have been similar although not identical to prevailing conditions in the test zone prior to the biostimulation experiments. During the first 11−13 days, all BTEX compounds increased in concentration, indicating desorption from the contaminated sediments (the data are summarized in ref 45). Then benzene, ethylbenzene, and *o*-xylene reached an apparent equilibrium, and their concentrations remained constant. In contrast, the concentrations of toluene and *m,p*-xylene began to decrease after 11−13 days, indicating selective depletion of these compounds. Figure 7 contrasts the concentration ratios of toluene/benzene and *m,p*-xylene/benzene with that of ethylbenzene/benzene from day 13 on. The data suggest selective removal of the toluene and *m,p*-xylene relative to benzene, consistent with laboratory results (*13*). The rates measured in the laboratory microcosms (at a solid to liquid ratio of approximately 1 g of sediment/5 mL of water) were much faster than those observed in the field where the solid to liquid ratio is approximately 5:1 (assuming a bulk density of 1.8 kg/L and a porosity of 33%). In the laboratory microcosms, 0.5 mg of toluene was removed at a constant (zero-order) rate in less than 20 days (a specific rate of approximately 0.8 μg of toluene (g of solids)$^{-1}$ day^{-1}. If the laboratory rates applied to in situ conditions, 1 mg of toluene should be removed from 1 L of groundwater in approximately 0.3 day (assuming that 1 L of groundwater is in contact with approximately 4.5 kg of sediment). The discrepancy between the slow removal under unamended conditions and the rapid removals observed in the laboratory is unexplained but could be due to slow mass transfer. Sulfate concentrations decreased from 100 mg/L initially to 80 mg/L toward the end of the test (data not shown) whereas nitrate increased from 0 to 4 mg/L during breakthrough of the groundwater. In EO2, where the injectate was not amended with nitrate and sulfate, some toluene removal was observed (data not shown).

BTEX Removal under Nitrate-Reducing Conditions. EO3 was the first of three experiments aimed at evaluating the effect of nitrate on BTEX removal. In EO3, the injectate was amended with 209 mg/L nitrate. Unexpectedly, the tracer response from EO3 did not reproduce the results from the two previous experiments, suggesting a change in the hydrodynamic conditions. One possible explanation was that N_2 was formed in excess of its solubility limit, leading to bubble

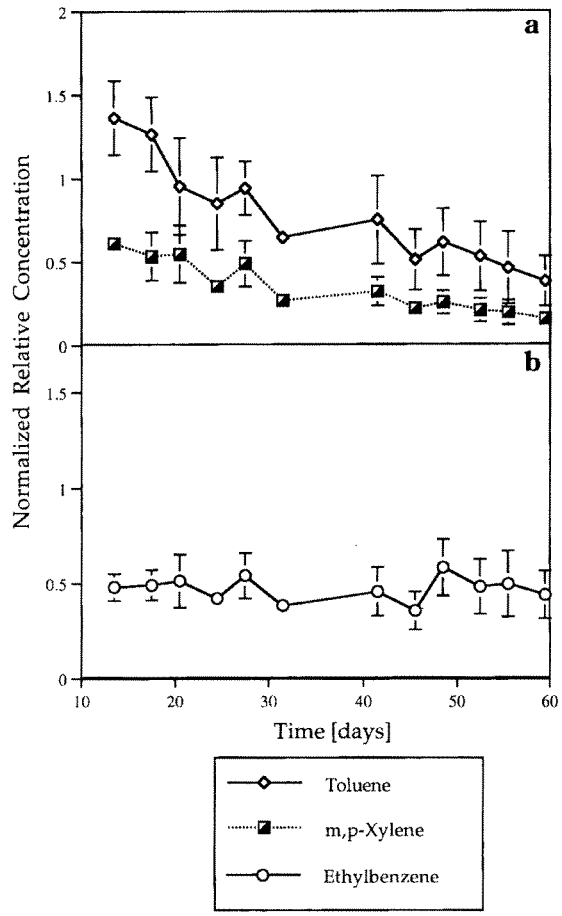

FIGURE 7. Average BTEX/benzene concentration ratios during EO1. The data are normalized to the highest observed values (observed on day 13). Concentrations of depths 2, 3, and 4 were averaged. Error bars indicate 1 SD.

formation and partial clogging. At the end of EO3, the concentrations of all BTEX compounds in the extraction water approached zero, perhaps because the BTEX compounds were degraded and/or flushed out.

In the following nitrate-amended experiment (EO4), the injectate was amended with benzene, toluene, ethylbenzene, *m*-xylene, and *o*-xylene along with bromide and nitrate, as indicated in Table 1. Figure 8 shows the observed response of BTEX compounds. Toluene and *m*-xylene were already removed to undetectable levels by day 10 when the first sample was taken. Ethylbenzene was completely removed by day 18. *o*-Xylene was removed to nondetectable levels by day 72 and followed the model response with a k_b of 0.1 μg L^{-1} h^{-1}. The apparent steady removal of *o*-xylene during EO4 was unexpected since laboratory studies suggested cometabolic removal in conjunction with the transformation of toluene. Benzene was stable, as expected based on laboratory results (*13*).

Test EO4 was repeated (EO5) to obtain better kinetic data for toluene, *m*-xylene, and ethylbenzene removal and to evaluate whether benzene transformation was inhibited by *o*-xylene. As shown in Figure 9, toluene and *m*-xylene were removed rapidly, followed by a slower removal of ethylbenzene. Benzene was again stable, even though *o*-xylene was absent. The removals of toluene, ethylbenzene, and *m*-xylene fit a zero-order model. Using eq 3 and k_b as a fitting parameter, the estimated rate constants were as follows: 4.5 μg L^{-1} h^{-1} for toluene and *m*-xylene, 2.8 μg L^{-1} h^{-1} for ethylbenzene, and 0.1 μg L^{-1} h^{-1} for *o*-xylene.

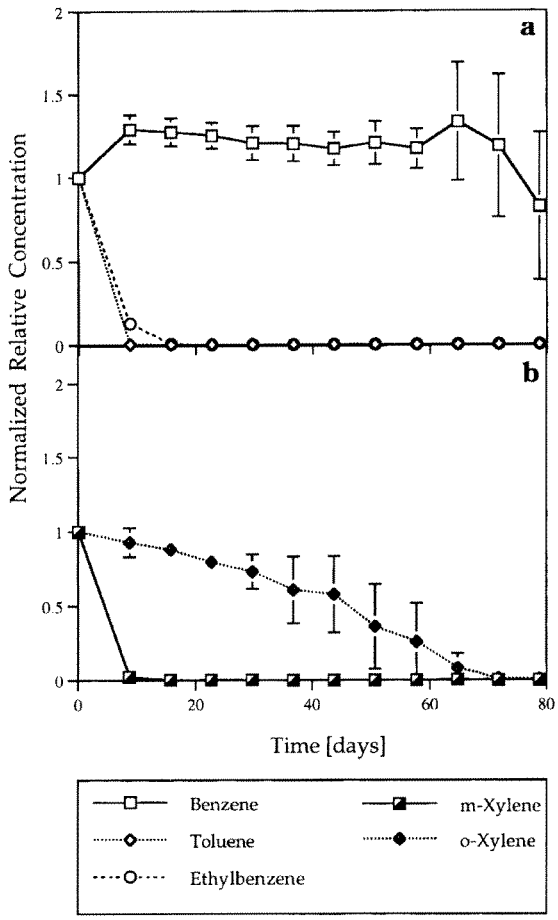

FIGURE 8. C/C_0 of BTEX concentrations normalized to Br/Br_0 during EO4 under nitrate-reducing conditions: (a) benzene, toluene, and ethylbenzene; (b): *m*-xylene and *o*-xylene. Symbols are the average of depths 2, 3, and 4; bars indicate 1 SD. For *o*-xylene, the line indicates model fit for $k_b = 0.1\ \mu g\ L^{-1}\ h^{-1}$.

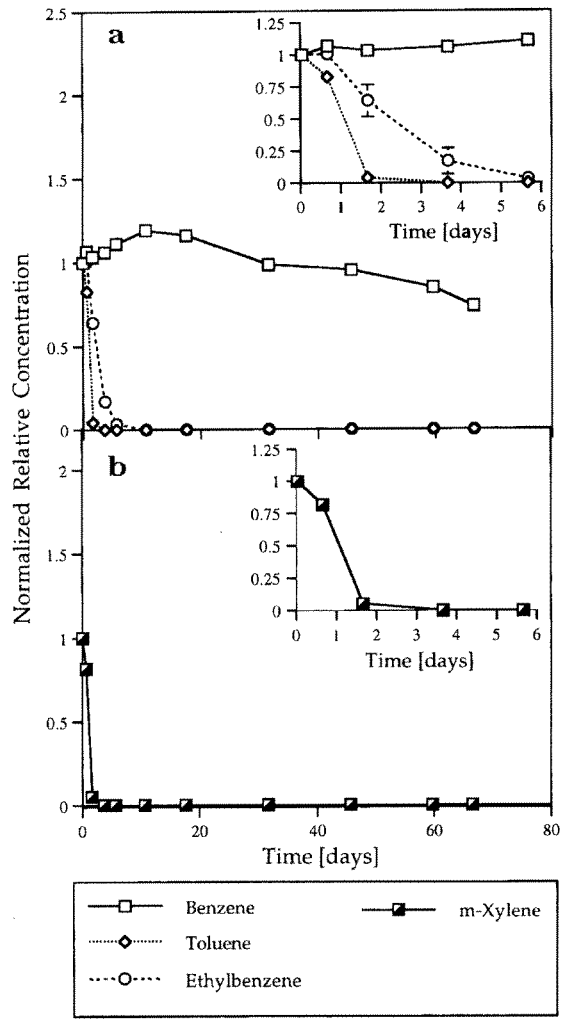

FIGURE 9. C/C_0 of BTEX concentrations normalized to Br/Br_0 during EO5 under nitrate-reducing conditions: (a) benzene, toluene, and ethylbenzene; (b) *m*-xylene. Lines connect the average values of depths 2, 3, and 4; bars indicate 1 SD.

The nitrate and BTEX utilized during EO4 and EO5 are summarized in Table 2. During EO4, the nitrate and BTEX removals in the composite sample were 62 and 0.47 mg/L, respectively. Data of the individual levels were generally within the range of the individual sampling levels (*45*). Assuming complete mineralization and no cell growth, the nitrate demand per milligram of BTEX is approximately 4.9 mg. Thus, the BTEX removed contributed at most 3.7% of the observed nitrate demand. In EO5, the nitrate demand was 15 mg/L, and BTEX removal contributed at most 15%. In both experiments, most of the nitrate was utilized during the first few days (*45*). Denitrification was indicated by the presence of nitrite, which increased to approximately 5 mg/L within the first sampling of EO4 and then decreased to 1 mg/L during the experiment (*45*). In EO5, nitrite was also detected, but concentrations were generally lower, exceeding 1 mg/L only in a few instances. Trace levels of oxygen in the influent (<0.15 mg/L) seem to have been immaterial and below the threshold for denitrification (*46, 47*).

BTEX Removal under Sulfate-Reducing Conditions. After the three nitrate-amended experiments (EO3–EO5), two controlled BTEX releases were conducted under sulfate-amended conditions (EO6 and EO7). The BTEX responses observed in EO6 and EO7 are shown in Figures 10 and 11, respectively. In EO6, toluene, *m*-xylene, and *o*-xylene were partially removed without a lag phase. Then, removal stopped for approximately 10 days before it resumed. Ethylbenzene showed similar behavior although the removal in the second phase was slower and incomplete. In EO7, the removals of toluene, *m*-xylene, and *o*-xylene were faster than in EO6 and were complete by day 46. The plateau observed in EO6 was not distinct in EO7. Removals of toluene and *m*- and *o*-xylene were consistent with zero-order kinetics. Again, removal of ethylbenzene was slower than the removals of toluene and the xylenes. Benzene concentrations showed a downward

TABLE 2. Comparison of Measured Nitrate and Sulfate Utilization with Observed BTEX Removal

expt	NO_3^- demand (mg/L)[a]	BTEX removed (mg/L)	theor. NO_3^- demand due to BTEX (% of total)
EO4	62 ± 12%	0.47	3.7
EO5	15 ± 23%	0.47	15
expt	**SO_4^{2-} (mg/L)**	**BTEX removed (mg/L)**	**SO_4^{2-} demand due to BTEX**
EO6	7.2 ± 22%	0.19	13
EO7	3.2 ± 16%	0.28	41

[a] RSTD, relative standard deviation determined from the average and standard deviation of the nitrate concentrations measured in all extracted samples during experiment.

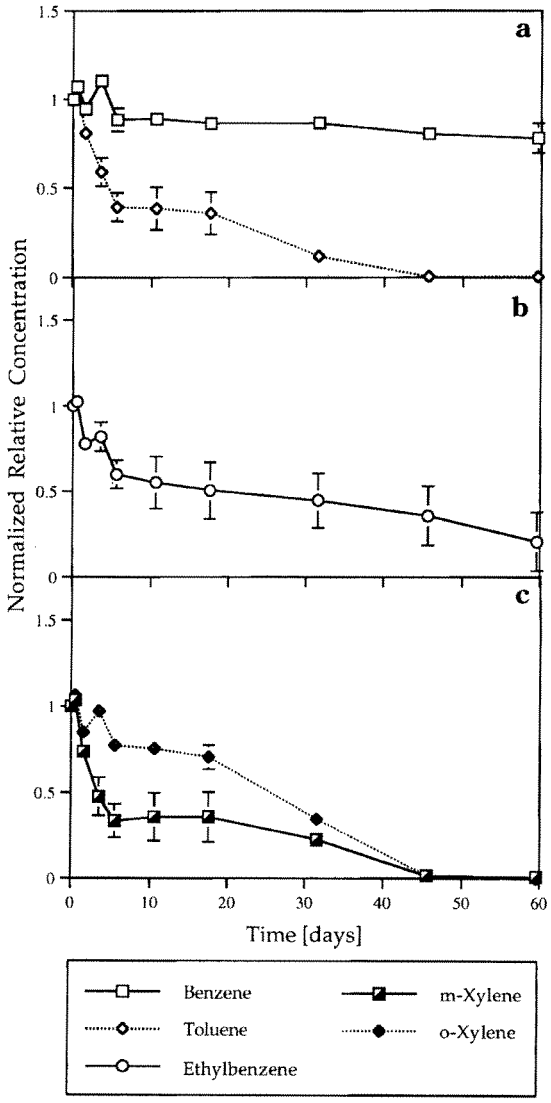

FIGURE 10. ***C/C₀*** **of BTEX concentrations normalized to Br/Br₀ during EO6 under sulfate-reducing conditions: (a) benzene and toluene; (b) ethylbenzene; (c) *m*- and *o*-xylene. Lines connect the average values of depths 2, 3, and 4; bars indicate 1 SD. If no error bars are shown, standard deviation is smaller than symbol.**

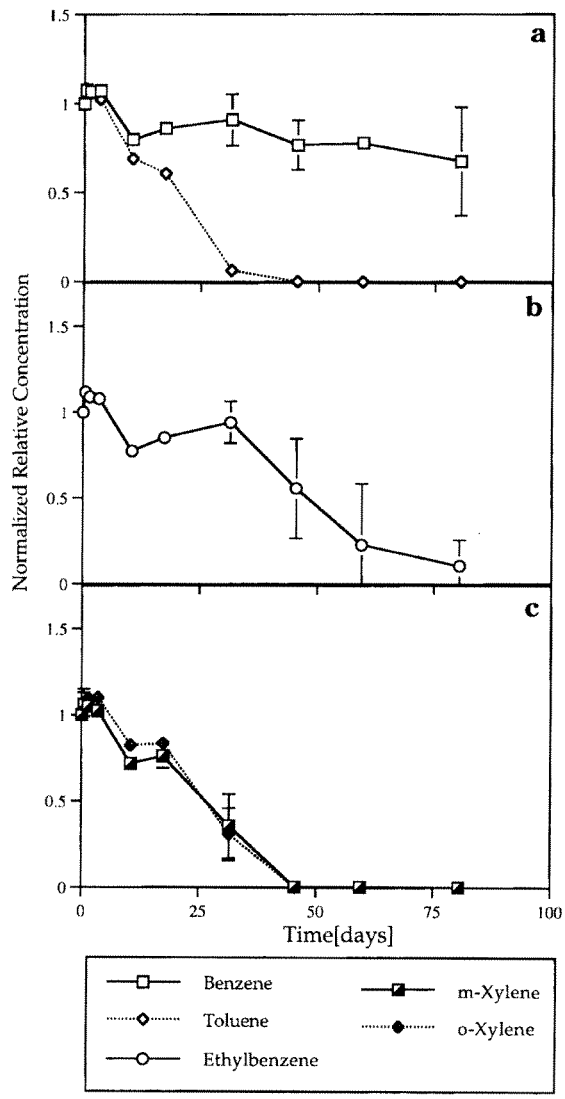

FIGURE 11. ***C/C₀*** **of BTEX concentrations normalized to Br/Br₀ during EO7 under sulfate-reducing conditions: (a) benzene and toluene; (b) ethylbenzene; (c) *m*- and *o*-xylene. Lines connect the average values of depths 2, 3, and 4; bars indicate 1 SD. If no error bars are shown, standard deviation is smaller than symbol.**

trend, but this trend was pronounced only at some depths (*45*). Long-term experiments are needed to ascertain biological benzene transformation.

Sulfate and BTEX utilization data obtained during the EO6 and EO7 are summarized in Table 2. In EO6, the sulfate and BTEX removed in the composite were 7.2 and 0.19 mg/L, respectively, and in EO7, removals for sulfate and BTEX were 3.2 and 0.28 mg/L, respectively. Assuming no cell growth and complete mineralization, the theoretical sulfate demand per milligram of BTEX is approximately 4.7 mg (*13*). Accordingly in EO6 and EO7, the BTEX contributed at most 13% and 40%, respectively, to the observed sulfate removals. In both cases, most of the sulfate removal occurred immediately after injection. The identity of the compounds other than BTEX that contribute to the sulfate demand is unknown but may have included other aromatic or partially oxidized organic compounds. It is unclear whether the concomitant utilization of these uncharacterized organic substrates affect the rate of BTEX transformation.

Sulfate-reducing conditions were indicated by the presence of small amounts of hydrogen sulfide. In EO6, hydrogen sulfide was detected in the composite samples after the two initial samplings. Concentrations ranged from the detection limit (0.03 mg/L) to 0.26 mg/L. In EO7, hydrogen sulfide concentrations were lower than in EO6 and never exceeded 0.135 mg/L (*45*). In all cases, the dissolved hydrogen sulfide measured was much less than the theoretically expected value; perhaps the sulfide sorbed onto or reacted with aquifer solids. A fraction of the hydrogen sulfide formed may have acted as a scavenger for trace levels of oxygen present in the injectate.

Microbial metabolism of toluene and *o*- and *m*-xylene was demonstrated by the detection of distinctive metabolic byproducts, as reported previously (*39*). During EO7, a strong correspondence was observed between the disappearance of toluene and *o*- and *m*-xylene from groundwater and the appearance of distinctive metabolic byproducts, such as benzylsuccinic, (2-methylbenzyl)succinic, and (3-methylbenzyl)succinic acid. EO7 was the only experiment during which samples for these metabolites were analyzed. The presence of these distinctive metabolic products is further

evidence that toluene and *m*- and *o*-xylene degraded anaerobically rather than aerobically (*39*).

In summary, the slug test methodology proved useful for the evaluation of microbial transformation of BTEX compounds in a slow moving aquifer. The methodology is suitable for aquifers with groundwater flow velocities on the order of 1 cm/day and processes that occur on time scales on the order of days to weeks. In general, the observed removals agreed with results from related laboratory studies although the rates of toluene and *m,p*-xylene removal under field conditions seemed slower than in related microcosms. The reasons for this apparent inhibition are not understood. The data of the controlled release experiments indicate that it is possible to enhance anaerobic bioremediation by inundating the test zone with ground water that has been deionized, GAC-treated, and augmented with nitrate or sulfate as the electron acceptor. Such enhancement may occur naturally at sites where groundwater flowing through the test zone constantly replenishes electron acceptors and removes potentially inhibiting products. Identifying the geochemical factors that control the rate of anaerobic BTEX biotransformation and quantifying their effects appear to be preconditions to taking full advantage of anaerobic bioremediation.

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