ground water

Spatial Variability of In Situ Microbial Activity: Biotracer Tests

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Abstract

Biotracer tests have been proposed as a means by which to characterize the in situ biodegradation potential for field-scale systems. In this study, field experiments were conducted at two sites to evaluate the utility of the biotracer method for characterizing the spatial variability of microbial activity. The first site is a mixed waste-contaminated surficial aquifer in Utah, and the second site is a chlorinated solvent-contaminated regional aquifer in Tucson, Arizona. Mass recovery of the biotracer decreased approximately linearly with increasing residence time for the Tucson site. Similar behavior was observed at the Utah site, except in the region adjacent to the injection zone, where percent recoveries were much lower than those predicted using a correlation determined using data collected downgradient of the injection zone. First-order biodegradation rate coefficients obtained from model calibration of the tracer data varied between 0.2 and 0.5/day for the Tucson site. For the Utah site, the values varied between 0.1 and 0.6/day downgradient of the injection wells, and between 0.7 and 2.6/day near the injection wells. Considering the large range over which biodegradation rate coefficients can vary, the rate coefficient exhibited relatively minimal spatial variability (factor of 2.5) for the Tucson site. Conversely, the spatial variability of the rate coefficient was an order of magnitude greater for the Utah site. These differences in variability are consistent with conditions associated with the respective sites. For example, the greater microbial activity observed in the vicinity of the injection wells for the Utah site is consistent with the biomass distribution determined from analysis of core samples, which shows larger bacterial cell densities for the region near the injection wells. These results illustrate the utility of biotracer tests for in situ characterization of microbial activity (e.g., biodegradation potential), including evaluation of potential spatial variability.

Introduction

In situ bioremediation has become a preferred method for cleaning up sites contaminated by organic compounds (National Research Council 1993; Macdonald and Kavanaugh 1994; Atlas 1995; Hart 1996). In situ bioremediation applications range from so-called intrinsic bioremediation, based on minimal energy and materials input, to more engineering-intensive techniques, which may include nutrient additions, oxygen sparging, or augmentation with exogenous microbial populations. In addition, the use of monitored natural attenuation, often synonymous with intrinsic bioremediation, is increasing in popularity (National Research Council 1993). Evaluating the feasibility of using in situ bioremediation at a site requires a determination of the in situ biodegradation potential for the target contaminants and the spatial distribution of this potential at the site.

Characterizing the magnitudes and rates of biodegradation at the field-scale is a complex task (Madsen 1991; Sims et al. 1993; McAllister and Chiang 1994). Methods for collecting such information at field-scale include monitoring parameters associated with biodegradation, such as contaminant concentration profiles, electron acceptor concentrations (such as O_2, NO_3^-, SO_4^{2-}, Fe^{3+}), contaminant metabolite concentration profiles, degradation products (CO_2, CH_4), and isotope ratios (Klecka et al. 1990; Borden et al. 1995; Chapelle et al. 1996). However, the complexity of typical field sites makes it difficult to correlate conclusively changes in these parameters to biodegradation. Furthermore, the initial mass of contaminant

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released into the subsurface is not known at most sites. Thus, it is difficult to quantify the magnitude and rate of biodegradation. Although mass balances have been obtained for controlled experiments conducted at a few field sites, allowing for the determination of in situ biodegradation rates (Barker et al. 1987; Roberts et al. 1990; Semprini et al. 1990; MacIntyre et al. 1993; Reinhard et al. 1997), this approach is not available for most contaminated sites.

Another approach often used to evaluate biodegradation potential is to conduct biodegradation studies in the laboratory using core samples collected from the field. However, given the sensitivity of microbial activity to extant conditions, results obtained from laboratory tests may not accurately represent field-scale behavior. In situ microcosms (ISM), in which tests are conducted in a small volume of the aquifer, can be used to minimize this problem (Gillham et al. 1990; Nielsen et al. 1996). Given the complexity of most field sites, including spatial variability of physical and chemical properties, and of microbial populations, a prohibitive number of sampling points may often be required to characterize fully a field site using either of these methods.

Biotracer tests have recently been proposed as an alternative method for field-scale characterization of the in situ biodegradation potential associated with subsurface microbial communities (Istok et al. 1997; Brusseau et al. 1997; Schroth et al. 1998; Brusseau et al. 1999a; Hageman et al. 2001). Biotracers may include any compound that is used by a microbial community including organic compounds, oxygen, nitrate, sulfate, etc. Biotracers that are structurally similar to specific contaminants present at the site may be used to evaluate the likelihood and potential rate of biodegradation of those contaminants by the indigenous microbial community (Brusseau et al. 1997, 1999a; Hageman et al. 2001). Furthermore, with appropriate sampling density, biotracer tests may be used to characterize the spatial distribution of microbial activity. Biotracer tests can also be used to evaluate the impact of potential perturbations, such as the addition of oxygen or a solubility-enhancement agent, on biodegradation.

In this study, field experiments were conducted at two sites to evaluate the utility of the biotracer method for characterizing spatial variability of microbial activity. The transport of biodegradable solutes was compared to that of bromide, a conservative, nonbiodegradable tracer. The transport data for these tracers were used to characterize the magnitude and rate of in situ biodegradation, and to evaluate the relationship between biodegradation and physical properties of the systems (residence time, spatial location).

Materials and Methods

Tracers

The selection of the biotracers involved several criteria. First, the tracers had to be of low toxicity to ensure approval from regulatory agencies for injection into the subsurface. In addition, to minimize problems with mass balance, mass transport, and bioavailability, it was desired that the compounds have low volatilities, large aqueous solubilities, and minimal sorption. Sodium benzoate, ethanol, pentanol, and hexanol met all these requirements. Initial column studies were performed using sterile soil to determine these compounds were not subject to abiotic transformations or sorption processes in either porous medium (Piatt 1997; Brusseau et al. 1999a). Another criterion was that the solutes had to be biodegradable under both aerobic and anaerobic conditions by commonly occurring subsurface microorganisms. These biotracers are all readily biodegraded under aerobic (Holder and Vaughan 1987; Grund et al. 1990; van Beilen et al. 1992; Altenschmidt et al. 1993; Wales and Fewson 1994; Nosova et al. 1997; Hirata et al. 2000) and anaerobic (Horowitz et al. 1983; Battersby and Wilson 1989; Kobayashi and Rittman 1982; van Iersel et al. 1997) conditions. Bromide (as $CaBr_2$) was used as the nonreactive, mass-conservative tracer.

An immiscible-liquid phase of organic contaminants is present at the Utah site (McCray and Brusseau 1998). Bromide, benzoate, and ethanol do not partition measurably to this phase. Conversely, pentanol and hexanol do partition measurably into the immiscible-liquid phase. Thus, the transport and biodegradation of pentanol and hexanol may be influenced by mass transfer between the aqueous and immiscible-liquid phases. Preliminary analysis of the transport of tracers that partitioned to the immiscible liquid, but experienced minimal biodegradation, indicates this mass transfer process can be considered to be essentially instantaneous with respect to hydraulic residence times prevalent in the system (unpublished data). Thus, when properly accounted for, the retention associated with partitioning to the immiscible liquid should not constrain determination of biodegradation rates for pentanol and hexanol.

Field Site 1: Utah

This field site, which resides in an area designated as operable unit 1, is located at Hill Air Force Base, Utah. As was noted, the site is contaminated with a multiple-component immiscible organic liquid. The contamination originates from multiple sources and is comprised primarily of jet fuel, along with chlorinated solvents and other hydrocarbons. The biotracer test was conducted in conjunction with a partitioning tracer test designed to measure the immiscible-liquid saturation present in the cell (Cain et al. 2000). The zone in which the tracer tests were conducted is part of an unconfined aquifer that consists of fine to coarse sand, interbedded with gravel and silt layers. Depth to the saturated zone ranges from 5 to 5.5 m. An isolated cell was created by driving sheet-piles into a clay unit located ~8 m bgs. The cell was ~3 by 5 m and contained four fully screened injection wells and three fully screened extraction wells. A 60-point, three-dimensional, multilevel sampling array (3 by 4 locations, five depths) connected to a vacuum system was used to collect depth-specific water samples (Figure 1). For this study, samples were collected from the 6.9 m depth array, as well as the extraction wells. Additional details of the site can be found in Cain et al. (2000).

Tracer-free water was first flushed through the cell for several days to create steady-state conditions. Peristaltic pumps (Cole Parmer Master Flex I/P with Tygon LFL tubing) with separate heads for each well were used to generate flow. The tracer pulse was then injected, followed by the

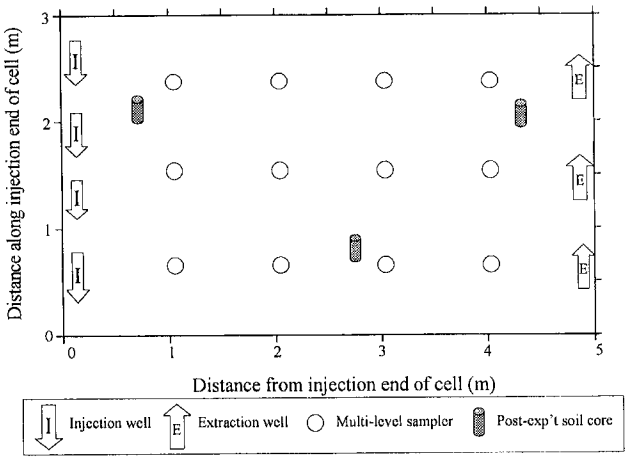

Figure 1. Schematic diagram of the flow cell developed at the Utah site. Locations of soil corings, injection wells, extraction wells, and multilevel sampling devices are shown.

injection of tracer-free water again. Constant flow rates of ~3.8 L/min (combined total for all injection and extraction wells, respectively) were maintained during the flow establishment, tracer injection, and water flushing portions of the experiment. Equal injection and extraction flow rates were maintained, with the flow divided evenly among the three extraction wells and four injection wells. A total tracer pulse of 1325 L was injected. The initial concentrations of the tracers injected into the cell were 304 mg/L for benzoate, 1081 mg/L for ethanol, 871 mg/L for hexanol, 797 mg/L for pentanol, and 300 mg/L for bromide. Collection of samples involved placing ~15 mL of ground water into 20 mL polyethylene vials containing a biocide. Samples were then refrigerated at 4°C and shipped overnight to the University of Arizona for analysis.

After the tracer test, cores were collected at three locations in the cell to enumerate general aerobic microbial populations and populations capable of aerobic degradation of jet fuel (JP8). The core locations corresponded to the injection end, middle, and extraction end of the cell (Figure 1). Cores were collected between the depths of 4 and 8 m bgs, which encompassed the saturated zone through which flow occurred. The core samples were refrigerated for a maximum of 24 hours until they were processed.

Two grams of soil for each sample were placed in 9 mL of autoclaved, deionized water (Basrnstead, Nanopure water, Dubuque, Iowa) and vortexed for one minute. The samples were then serially diluted to a range that would produce statistically robust microbial counts. The dilutions were plated on plates containing R_2A (Becton and Dickinson Company, Cockeysville, Maryland) or mineral salts medium (MSM) combined with 0.05% JP8 (added after autoclaving). MSM contains 0.4% Na_2HPO_4, 0.1% KH_2PO_4, 0.1% NH_4Cl, 0.2% $MgSO_4$, 0.005% yeast extract, 0.0005% ammonium iron (III) citrate, 0.001% $CaCl_2$, and 15 g agar. The R_2A media provides a measure of heterotrophic bacteria while the MSM/JP8 media provides a measure of bacteria capable of degrading jet fuel components. Samples were plated in triplicate, along with appropriate controls. The plates were incubated at room temperature for one week and were then counted.

Prior to the biotracer test, a 10% cyclodextrin solution was pumped through the aquifer for 10 days (a total of 65,500 L), followed by flushing with water free of cyclodextrin (~40,000 L). This pumping activity was associated with a pilot test of an enhanced-solubilization remediation technology (McCray and Brusseau 1998). During this test, ~100% of the injected cyclodextrin was recovered, indicating that degradation of this compound was negligible. The pumping introduced aerated water into the cell for a significant period of time (> 15 days). The oxygen concentration in the injected water was ~8 mg/L, while it was 1 to 2 mg/L in the effluent pumped from the extraction wells. In addition, the cyclodextrin solution was shown to have enhanced the solubilization and removal of organic constituents (McCray and Brusseau 1998). This enhanced-solubilization process may have increased the bioavailability of the organic contaminants, as was demonstrated in prior laboratory experiments (Wang et al. 1998). Thus, it is likely that the injection of the aerated cyclodextrin solution stimulated microbial activity within the cell, which in turn resulted in the measured increase in biomass near the injection wells (Figure 2).

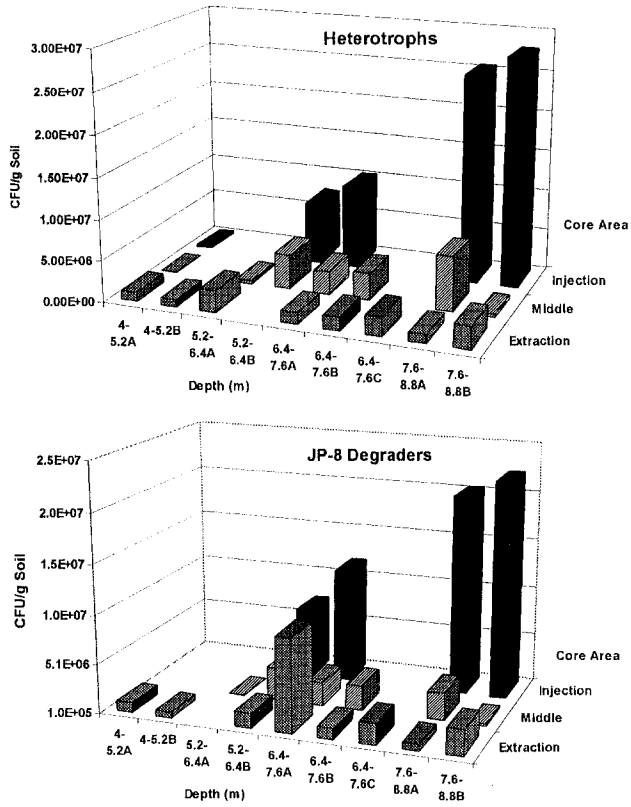

Figure 2. Microbial distribution for the Utah field site determined from analysis of subsamples obtained from three cores. Viable counts for total heterotrophs and JP8 degraders are shown. The letters attached to the core depths represent the location from which the sample was collected, with sample A collected near the top of the core and sample B collected near the bottom. For the 6.4–7.6 core, samples A, B, and C were collected from the top, middle, and bottom of the core, respectively.

Field Site 2: Tucson, Arizona

This trichloroethene-contaminated field site is part of the Tucson International Airport Area Superfund site in Tucson, Arizona. The tracer experiment was conducted in a semiconfined, 6 m thick aquifer located 36 m bgs. Analysis of core material indicates the aquifer is comprised primarily of sand, with some clay lenses and cobbles. An organic carbon content of 0.03% was measured for several core subsamples. A single injection-extraction well couplet, separated by ~57 m, was used for this experiment. The tracer solution, comprising benzoate and bromide (45 mg/L each), was injected into the subsurface at 170 L/min for 56 hours for a total pulse of 620,000 L. After injection of the pulse, tracer-free water was continuously injected until the end of the experiment. Aqueous-phase samples were collected from an array of 10 monitoring wells screened across the entire 6 m thickness of the aquifer. Samples were collected using a stainless steel bailer. The tracer concentrations obtained for these locations represent depth-averaged values. In addition, an eleventh well was equipped with a four-port, multilevel sampler (MLS) to collect depth-specific samples. The four sampling ports were spaced across the 6 m thickness of the aquifer. Additional details regarding the field site are available in Brusseau et al. (1999b).

~10 mL of ground water was collected into 20 mL polyethylene vials with screw caps. The samples were autoclaved and stored under chilled conditions until analysis to prevent degradation of benzoate in the vials. Laboratory studies showed that autoclaving does not affect the concentration of benzoate in the samples (i.e., no occurrence of thermal degradation, volatilization, or concentration due to evaporation of water).

Laboratory Column Experiments

Batch and column experiments were conducted to ensure the selected biotracers could be biodegraded by the resident communities, and that bromide was not. In addition, column experiments were conducted to determine the applicability of the simple, first-order based biodegradation model to systems with small input pulses that may be experiencing bacterial growth. For these experiments, aquifer material was collected from both sites using aseptic techniques. The experiments were conducted following previously described methods (Brusseau et al. 1999c; Sandrin et al. 2001).

Chemical Analysis

Prior to analysis, the samples were filtered through 0.2 μm syringe filters to remove particulate matter. The filtered samples for benzoate detection were transferred directly to 1 mL glass autosampler vials and capped with starburst snap plugs. Samples were analyzed with an automated HPLC system (Waters) using a mobile phase of 40% acetonitrile and 60% pH 3.6 phosphate buffer (0.01 M) and an Adsorbosphere UHS C18 column, and detected using a UV-Vis spectrophotometer (λ = 235 nm). The quantifiable detection limit for benzoate was ~500 μg/L. The alcohols were analyzed using a gas chromatograph (Shimadzu GC–14A) equipped with a flame ionization detector, with a resultant quantifiable detection limit of ~500 μg/L. Bromide was analyzed using a colorimetric technique (Alpkem), with a quantifiable detection limit of ~300 μg/L.

Data Analysis

Mass recoveries and travel times for the tracers were calculated by temporal moment analysis of the breakthrough curves. The zeroth moment is a measure of the mass recovered for a given tracer:

$$M_o = \int C dt$$

The first moment is a measure of the mean arrival of the solute pulse:

$$M_1 = \int C t dt$$

The mean travel time (MTT) is the time required for the mean position of the tracer pulse to move from the injection well to a given monitoring location:

$$MTT = M_{1,norm} - 0.5T_o$$

where $M_{1,norm}$ is M_1 divided by M_o, and T_o is the pulse-injection time normalized by the MTT of the nonreactive tracer. The percent recovery of the tracers was calculated as the ratio of the mass of the tracer recovered (M_o) to the mass of the tracer injected (the product of the pulse width, the injection flow rate, and the concentration).

The breakthrough curves obtained from the tracer test conducted at the Tucson field site were analyzed using the standard advection-dispersion transport equation. The values of the Peclet number were determined using a nonlinear least-squares optimization program incorporating the advection-dispersion equation. Generally, the Peclet number is defined as P = vL/D, where v is the average linear pore-water velocity (L T^{-1}), L is characteristic system length (L), and D is the hydrodynamic dispersion coefficient (L^2 T^{-1}). The dispersion coefficient is defined as $D = (D_0/\tau) + \alpha_L v$, where D_0 is the aqueous diffusion coefficient (L^2 T^{-1}), τ is the tortuosity factor, and α_L is the longitudinal dispersivity (L). For larger velocities (e.g., v >1 cm/hr), the D_0/τ term is usually considered negligible and the dispersion coefficient reduces to $D = \alpha_L v$.

In some cases, the degree of nonideal transport (e.g., spreading, tailing) is such that the standard advection-dispersion transport equation cannot provide a satisfactory simulation of observed breakthrough curves. This was the case for the results obtained from the tracer test conducted at the Utah field site. Thus, the breakthrough curves obtained for this system were analyzed using a model based on the dual-porosity conceptualization. This approach has been widely used to simulate solute transport in structured and layered systems including small field-scale systems. The dual-porosity concept serves as a useful, albeit simplistic, approach to incorporate the impact of physical heterogeneity on solute transport. For the dual-porosity model, the following two parameters were optimized in addition to P:

$$\omega = \alpha_{mt} L/q$$

$$\beta = \theta_a/\theta$$

where α_{mt} is the first-order mass transfer coefficient (T^{-1}), q is Darcy velocity (L T^{-1}), θ_a is the fraction of porosity in

which advection occurs, and θ is the porosity. For this model, the Peclet number represents local-scale spreading phenomena, whereas the ω parameter, which is the ratio of the effective hydraulic residence time to the characteristic time of mass transfer, represents the impact of physical heterogeneity (preferential flow, interregion diffusive mass transfer) on transport. The β parameter indicates the relative fraction of the domain for which advective flux is significant.

The nonreactive tracer data were calibrated with the model to determine values for P, β, and ω. First-order biodegradation rate coefficients were then determined for the biotracers by calibrating a model incorporating first-order biodegradation, coupled to a nonlinear, least-squares optimization routine, to the measured biotracer breakthrough curves. For these calibrations, X_c, the nondimensional degradation constant, and R (retardation factor) were optimized. The retardation factor is defined as

$$R = 1 + K_n\theta_n/\theta_w$$

where K_n is the immiscible-liquid/water partition coefficient, θ_w is volumetric water content, and θ_n is volumetric immiscible-liquid content. For ethanol and benzoate, values for R were fixed at 1, given that these two tracers do not partition measurably to the immiscible liquid.

The nondimensional degradation constant is defined as

$$X_C = \frac{\mu L}{v}$$

where μ (T^{-1}) is the first-order biodegradation rate coefficient. The influence of microbial properties on the magnitude of μ can be evaluated in terms of the Monod equation, which is widely used to describe microbial growth and substrate biodegradation:

$$\mu \approx \frac{\mu_m B}{Y(K_S + C)}$$

where μ_m is the maximum specific growth rate (T^{-1}), B is the biomass concentration (M L^{-3}), Y is the yield coefficient, and K_S is the half-saturation coefficient. When the concentration of substrate is much larger than the half-saturation constant, as is likely for the tracer tests reported herein (due to the large C_0 values), $\mu = \mu_m B/Y$. The biomass concentration at a field site may vary by orders of magnitude and, thus, is generally expected to vary to a much greater extent than μ_m or Y. Thus, the spatial variability of μ is expected to be directly influenced by spatial variability of biomass concentrations. The validity of using a linear biodegradation model for the tracer data was evaluated as will be discussed later.

Contour plots were constructed to depict spatial variability of travel times and biodegradation rate coefficients at the Utah site. These plots were constructed in the same format and on the same scale as the Utah site map (Figure 1). Travel times and rate coefficients were determined for each sampling and extraction well location. Kriging interpolation techniques were used to develop the contours.

Results and Discussion

Laboratory Studies

Batch biodegradation studies using aquifer material from both sites confirmed that the indigenous microflora was capable of degrading the biotracer compounds used in this study. Other mass loss processes such as volatilization, sorption, and hydrolysis were negligible for these systems. Thus, it appears reasonable to assume that the additional mass loss observed for the biotracers compared to the nonreactive tracer during the field tests is due to biodegradation. Conversely, bromide was not degraded. The results of column experiments conducted using sterilized aquifer material showed that sorption of the tracers is negligible. The results of column experiments conducted using nonsterilized material indicated the general transport and biodegradation behavior of the biotracers were similar to that of representative site contaminants. For example, the rate and magnitude of biodegradation of benzoate during transport were very similar to that of toluene. The results of these laboratory experiments are discussed in Brusseau et al. (1999a).

Results from laboratory column experiments were used to test the applicability of the linear, first-order biodegradation model for analyzing the results of the field tests. Columns were packed with aquifer material collected from the Utah site and subjected to toluene inputs of two different pulse lengths. The results obtained for the long input-pulse (~32 pore volumes) experiment indicated that significant microbial growth occurred during the experiment (data not shown). The large growth precluded the use of the first-order degradation model. Conversely, the breakthrough curve obtained for the small input-pulse (~0.3 pore volumes) experiment, for which there was minimal growth, was fitted well by the model based on first-order degradation (data not shown). The field biotracer tests were conducted with short input pulses, 0.15 to 0.63 pore volumes, depending on the distance of the sampling location from the injection well. Therefore, microbial growth is expected to be relatively low during the test. Thus, the application of the linear biodegradation model to the tracer tests should produce reasonable results.

Site 1: Utah

Representative breakthrough curves of the tracers are shown for two sampling locations, one near the injection wells and one representative of the extraction wells (Figure 3). The arrival fronts for benzoate and ethanol are generally coincident with the arrival front for bromide. Conversely, pentanol and hexanol transport, due to partitioning to the immiscible-liquid phase, is retarded with respect to the other tracers. Bromide has a higher peak than that of the biotracers, and exhibits longer elution tails. This behavior is due to the biodegradation and resultant mass loss of the biotracers. The mathematical model provides a reasonable simulation of the measured breakthrough curves (Figure 3). However, the measured elution tails for the biotracers are generally shorter than the simulated curves, which suggests there may have been some increase in biodegradation potential during the tracer test. The mean optimized values

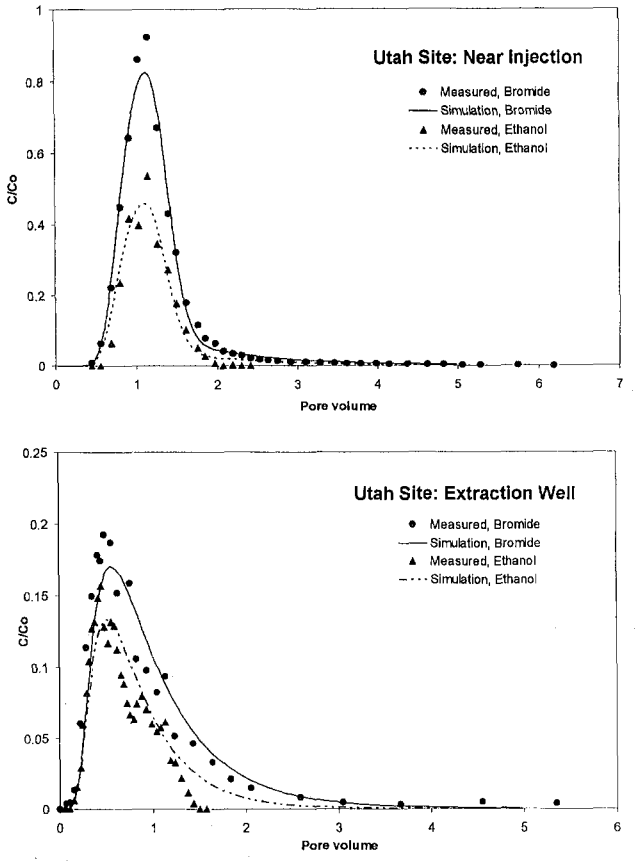

Figure 3. Representative breakthrough curves and simulation results for two sampling locations at the Utah field site.

for P, β, and ω, which were obtained from calibration of the bromide data, were 20, 0.67, and 0.2, respectively.

Mass recoveries for bromide ranged from 80% to 112%, reflecting the influence of hydraulic-related factors on transport. The recoveries of the biotracers were less than, or equal to, the recoveries of bromide for all monitoring locations (Figure 4), thus indicating the impact of microbial activity (given the absence of abiotic loss processes). Mass loss of the biotracers was observed for almost all sampling locations. Given that oxygen concentrations were 1 to 2 mg/L in the extraction well effluent, these results suggest that both aerobic and anaerobic degradation processes may have contributed to biotracer mass loss. This is consistent with the results obtained from the laboratory experiments.

The percent recovery for all of the biotracers is approximately linearly related to the residence time of the compound in the system when data points near the injection wells are omitted (Figure 4). The impact of residence time on the magnitude of biodegradation is well established, with greater mass loss expected for larger travel times. These results indicate that residence time is the primary factor influencing the differences in mass recovery for the biotracers, except in the vicinity of the injection wells. While percent recovery correlates well with residence time, it does not correlate well to distance from the injection wells. This is due to the spatial variability of travel times, a function of physical heterogeneities in the cell. The extent of the heterogeneity is apparent from inspection of the contour plot describing the travel time distribution of the conservative tracer within the cell (Figure 5). While travel times are generally larger at the extraction end of the cell, there are regions corresponding to larger travel times near the injection wells, presumably due to the presence of lower-permeability zones.

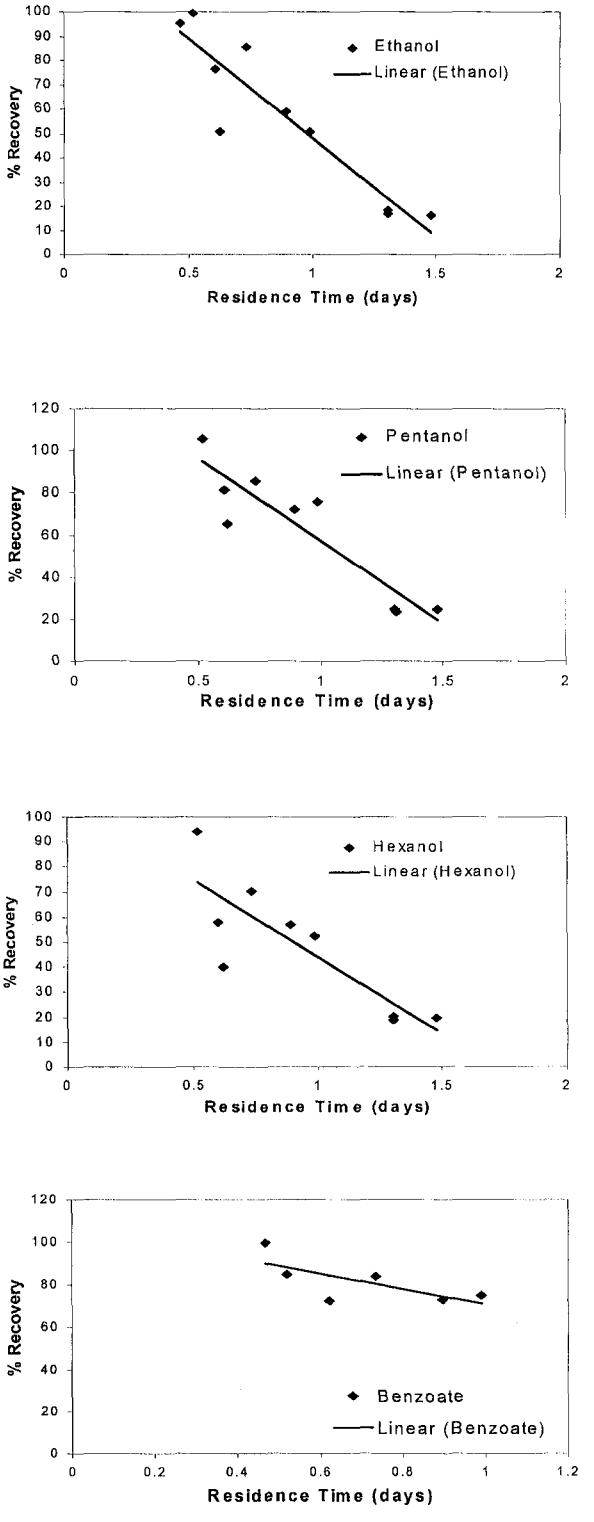

Figure 4. The percent of mass recovered for the biotracers used at the Utah site as a function of residence time. Data obtained from sampling locations near the injection wells are excluded. (a) ethanol, R^2 = 0.86, (b) pentanol, R^2 = 0.83. (c) hexanol, R^2 = 0.70. (d) benzoate, R^2 = 0.51.

A plot of the first-order biodegradation rate coefficient (μ) vs. bromide travel times is presented in Figure 6. The rate coefficient is poorly correlated to residence time (correlation

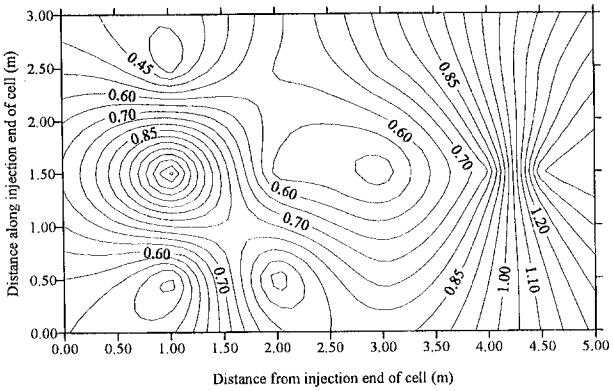

Figure 5. Spatial distribution of travel times for the conservative tracer (bromide) at the Utah site. The left side of the figure corresponds to the injection well locations. Units are in days.

coefficient < 0.33). The spatial distributions of the biodegradation rate coefficients are shown with contour plots presented in Figure 7. The values vary between 0.7 and 2.6/day near the injection wells, and between 0.1 and 0.6/day downgradient of the injection wells. As discussed previously, the Utah site was subjected to a cyclodextrin flush prior to the tracer test. This flush enhanced mixing in the cell, increased the solubilization of lower-solubility contaminants, and infused regions near the injection wells with oxygen, thereby stimulating microbial growth. This growth increased the biomass in the vicinity of the injection wells. Specifically, the bacterial cell densities were an order of magnitude higher, in terms of both heterotrophic and JP8 degrader counts, near the injection wells compared to the remainder of the cell (Figure 2). This larger biomass concentration is most likely responsible for the larger first-order biodegradation rate coefficients obtained for the region near the injection wells.

Site 2: Tucson

A representative set of breakthrough curves obtained from the tracer test conducted at the Tucson site is shown in Figure 8. Recoveries for bromide ranged between 80% and 103%. For all monitoring locations, the recovery of benzoate was less than the recovery of bromide, and the concentrations of benzoate returned to detection limits

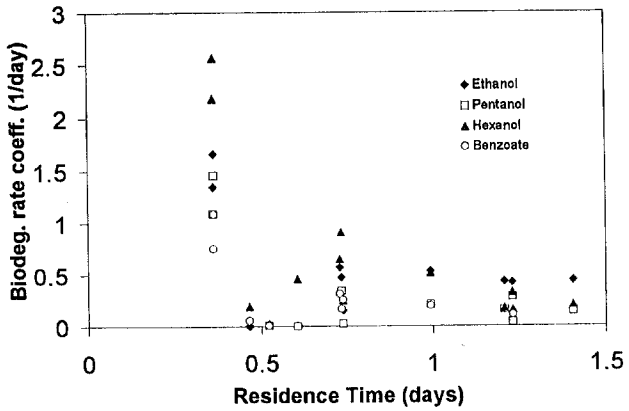

Figure 6. The relationship between biodegradation rate coefficient and residence time for the Utah site.

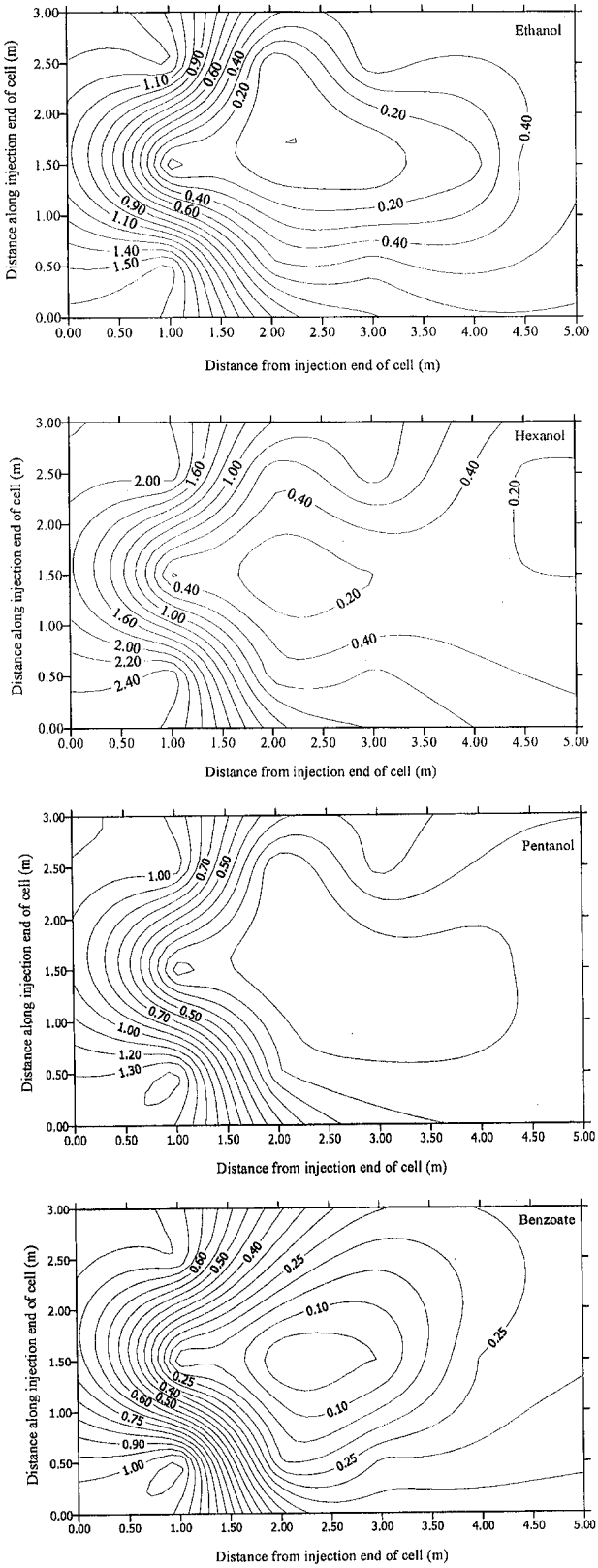

Figure 7. The spatial distribution of biodegradation rate coefficients at the Utah site. The left sides of the figures correspond to the injection well locations. Units are in days^{-1}.

much earlier than did bromide. The mass recoveries for benzoate ranged from 14% to 81%, and they decreased with increased residence time (Figure 9). The mathematical model provided reasonable simulations of the measured data. The mean optimized value for P was 7.

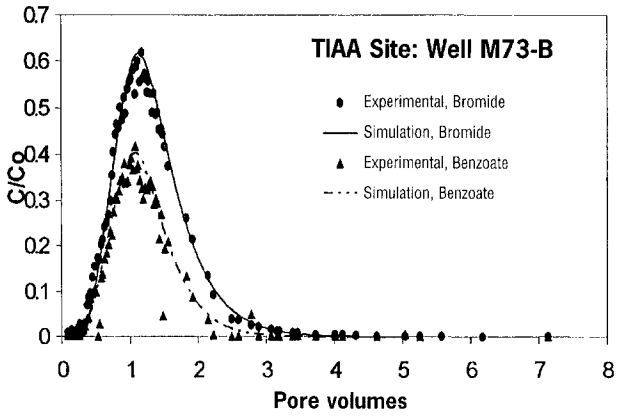

Figure 8. Representative breakthrough curves and simulation results for the Tucson field site.

The biodegradation rate coefficients were relatively similar throughout the Tucson site, ranging from 0.2 to 0.5/day, despite the presence of significant physical heterogeneity in the subsurface. This small range of values most likely reflects a relatively uniform distribution of microbial biomass. This would be consistent with the generally low level of inherent microbial activity at the site due to low dissolved oxygen concentrations, which are in the range of 1 to 3 mg/L, and the low organic carbon levels. The spatial variability of the rate coefficient was an order of magnitude greater for the Utah site, reflecting the greater level of microbial activity at that site.

The magnitudes of the biodegradation rate coefficients obtained for the two field tests are approximately one to two orders of magnitude larger than the rate coefficients reported for benzene, p-xylene, naphthalene, and o-dichlorobenzene for a natural-gradient field experiment conducted at Columbus Air Force Base (MacIntyre et al. 1993). They are also an order of magnitude larger than the values reported for toluene obtained from analysis of a contaminant plume located at a site in South Carolina (Chapelle et al. 1996). In addition to the expected differences in microbial communities, the observed differences in rate coefficients may reflect, at least in part, the different compounds used in the various studies. Both inherent rates of metabolism as well as the relative impact of bioavailability constraints may differ between the biotracers used herein and the compounds used in the other studies.

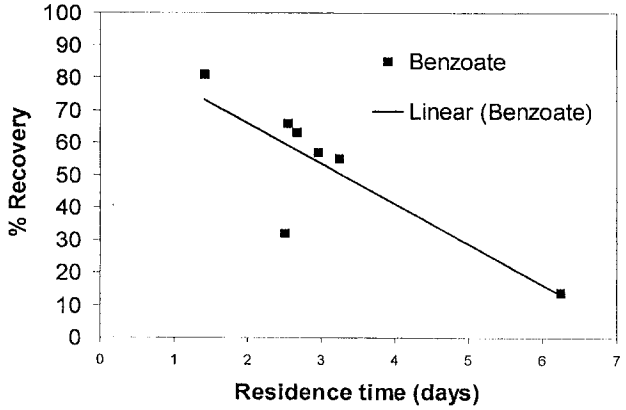

Figure 9. The percent of mass of benzoate recovered as a function of residence time for the Tucson site. The correlation coefficient for the linear least-squares fit is 0.69.

Conclusions

The results of the laboratory and field experiments indicate that the biotracers were, in fact, biodegraded and that the extent of mass loss was generally correlated to residence time (except in the vicinity of the injection wells for the Utah site). The biodegradation rate coefficient exhibited relatively minimal spatial variability (factor of 2.5) for the Tucson site. Conversely, the spatial variability of the rate coefficient was an order of magnitude greater for the Utah site. These differences in variability are consistent with conditions associated with the respective sites.

Microbial distributions in the subsurface are influenced by a number of environmental factors, such as carbon, oxygen, or nutrient availability; pH; and soil texture. Nonuniform spatial distributions of bacteria in the subsurface as a function of these factors has been reported by a number of researchers (Harvey et al. 1984; Harvey and Barber 1992; Kieft et al. 1998; Krueger et al.1998; Stapleton et al. 2000). Additionally, the impact of spatially variable microbial parameters has been demonstrated to significantly influence contaminant transport and biodegradation behavior (Miralles-Wilhelm and Gelhar 2000). Despite the importance of characterizing the spatial variability of microbial populations and activities, this is rarely done for field sites. This lack of characterization is due, at least in part, to constraints associated with traditional characterization techniques, many of which are labor intensive and/or limited by spatial variability.

The results of these experiments illustrate the potential of biotracer tests to evaluate the biodegradation capability of indigenous communities, and to characterize the spatial distribution of microbial activity or biodegradation potential at a site. With appropriate tracer selection, biotracer tests can be used to evaluate the biodegradation potential of specific contaminants of interest. Clearly, however, the behavior measured for the biotracers may not always be directly scalable to that of target contaminants. The relationships may change as a function of several factors, including the microbial community that is present at a particular site. Furthermore, additional research is necessary to evaluate the ability of biotracer-test methods to characterize the impact of bioavailability constraints on in situ biodegradation. Thus, the application of biotracer tests should be tempered by these potential constraints.

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