Environ. Sci. Technol. **2002**, *36,* 2262−2268

Stable Carbon Isotope Fractionation during Enhanced In Situ Bioremediation of Trichloroethene

DONALD L. SONG,[†,‡] MARK E. CONRAD,[§] KENT S. SORENSON,[⊥] AND LISA ALVAREZ-COHEN*[,†]

Department of Civil and Environmental Engineering, 631 Davis Hall, Berkeley, California 94720-1710, Center for Isotope Geochemistry, MS 70A-4418, E. O. Lawrence Berkeley National Laboratory, Berkeley, California 94720, and North Wind Environmental, Inc., P.O. Box 51174, Idaho Falls, Idaho 83405

Time-series stable carbon isotope monitoring of volatile organic compounds (VOCs) at the Idaho National Engineering and Environmental Laboratory's (INEEL) field site Test Area North (TAN) was conducted during a pilot study to investigate the treatment potential of using lactate to stimulate in situ biologic reductive dechlorination of trichloroethene (TCE). The isotope ratios of TCE and its biodegradation byproducts, *cis*-dichloroethene (*c*-DCE), *trans*-dichloroethene (*t*-DCE), vinyl chloride (VC), and ethene, in groundwater samples collected during the pilot study were preconcentrated with a combination of purge-and-trap and cryogenic techniques in order to allow for reproducible isotopic measurements of the low concentrations of these compounds in the samples (down to 0.04 μM, or 5 ppb, of TCE). Compound-specific stable isotope monitoring of chlorinated solvents clearly differentiated between the effects of groundwater transport, dissolution of DNAPL at the source, and enhanced bioremediation. Isotope data from all wells within the zone of lactate influence exhibited large kinetic isotope effects during the reduction of *c*-DCE to VC and VC to ethene. Despite these large effects, the carbon isotope ratio of ethene in all these wells reached the carbon isotope ratios of the initial dissolved TCE, confirming the complete conversion of dissolved TCE to ethene. Conversely, the carbon isotope ratios of *t*-DCE were only marginally affected during the study, indicating that minimal biologic degradation of *t*-DCE was occurring.

Introduction

Chlorinated solvents such as TCE are widespread groundwater contaminants that are suspected human carcinogens (*1*). Remediation of these pollutants is often hindered by the fact that these compounds are present as dense nonaqueous phase liquids (DNAPLs) at many sites, limiting the effectiveness of conventional pump-and-treat remedial strategies (*2, 3*).

In situ bioremediation, utilizing enhanced or natural biotransformation of pollutants, has emerged as a viable complement or alternative to conventional methods of remediating sites contaminated with chlorinated solvents (*4*). A promising strategy for in situ bioremediation of chlorinated solvents utilizes the process of microbial reductive dehalogenation (*5*). For TCE, this involves the sequential reduction of TCE to DCE (primarily the *cis*-1,2-DCE isomer, but *trans*-1,2-DCE and 1,1-DCE have also been observed), followed by conversion of DCE to VC, and finally VC to ethene. The microorganisms that catalyze these reactions use the solvents as electron acceptors while using a range of substrates (e.g., lactate, methanol, H_2) as electron donors under anaerobic conditions. Bacteria execute this transformation pathway as a cometabolic process or, in the case of halorespiring bacteria, as an energy-generating process using the chlorinated solvents as terminal electron acceptors (*6*). Reductive dechlorination is thought to be responsible for the natural attenuation of chlorinated solvents at sites with suitable environmental conditions (e.g., refs *6*−*11*).

The most serious issue associated with the use of reductive dechlorination as a remediation option is that the process does not always continue to ethene. This can lead to accumulation of DCE and VC, which is of great concern because both are hazardous compounds and VC is a known human carcinogen. Such incomplete dechlorination is commonly observed at field sites where reductive dechlorination of TCE is taking place (*6*).

This problem underscores the importance of an effective monitoring program at sites where reductive dechlorination is being utilized to remediate chlorinated solvents. However, monitoring the progress of in situ microbial reductive dechlorination is an especially difficult challenge. Distinguishing between concentration changes of the contaminants due to biodegradation rather than physical processes such as groundwater transport and mixing is a critical issue given the toxicity of the intermediate products. In addition, VC and ethene are substantially more volatile and less prone to sorption by organic matter than TCE and DCE, further hindering quantification (*12*−*15*). Thus, because these physical and chemical fate and transport processes complicate monitoring, complete stoichiometric conversion of TCE to ethene can rarely be demonstrated in the field.

Compound-specific stable carbon isotope analysis represents a powerful monitoring tool to provide insight into the fate and transformation of chlorinated solvents in the subsurface (*16*−*18*). Transformation of organic compounds by biological enzymatic processes can cause significant shifts in the ratio of ^{13}C to ^{12}C ($\delta^{13}C$ values) in both the reactants and products. This phenomenon occurs because of the stronger molecular bonds (with higher activation energies) formed by ^{13}C in comparison to ^{12}C and is referred to as *kinetic isotope fractionation* (*19*). Hence, molecules with the lighter isotopes tend to be transformed more quickly, resulting in enrichment of ^{13}C (increased $\delta^{13}C$) in the residual reactant and of ^{12}C (decreased $\delta^{13}C$) in the initial product. However, if a finite amount of reactant is present and the reaction proceeds to completion, then the isotope ratio of the product will equal that of the initial reactant. Therefore, it is only during the incomplete transformation that the isotope ratios of both reactants and products are transiently affected.

An early study with chlorinated solvents suggested the possibility of using compound-specific stable isotopes as conservative tracers for source characterization (*20*). This study presented data on the chlorine and carbon isotope

* Corresponding author phone: (510) 643-5969; fax: (510) 642-7483; e-mail: alvarez@ce.berkeley.edu.
[†] Department of Civil and Environmental Engineering.
[‡] Present address: Brown and Caldwell, 201 North Civic Drive, Walnut Creek, CA 94596.
[§] E. O. Lawrence Berkeley National Laboratory.
[⊥] North Wind Environmental, Inc.

10.1021/es011162d CCC: $22.00
Published on Web 04/06/2002

ratios of PCE, TCE, and trichloroethane from different manufacturers and found them to be isotopically distinct. In fact, several recent studies with chlorinated solvents have shown the fractionation of carbon isotopes during mass transfer processes such as sorption, dissolution, and volatilization to be quite small (*21*−*25*). However, recent studies conducted with laboratory microcosms have indicated that the microbial reductive dehalogenation of TCE results in significant kinetic fractionation of the carbon isotope ratios of TCE and intermediates *c*-DCE, VC, and the final product ethene (*16*, *17*, *26*). These studies found that the magnitude of isotopic fractionation increased with each dechlorination step, such that the observed fractionation was relatively small for the transformation of TCE to *c*-DCE when compared to the fractionation for the transformation of VC to ethene. Abiotic zerovalent iron reduction of TCE has also been shown to produce a kinetic fractionation effect similar in magnitude to those observed for biological reductions (*27*, *28*).

Several recent field studies have used stable isotopes to evaluate the intrinsic bioremediation of chlorinated ethenes. Sturchio et al. (*29*) measured shifts in the chlorine isotope ratios of bulk chlorinated solvents and chloride in groundwater from an industrial site to evaluate the potential for aerobic natural attenuation of TCE. They found small shifts in the chlorine isotope ratios of the chlorinated solvents along the gradient of the TCE plume that suggested natural attenuation may be occurring, but that interpretation was somewhat confounded by the lack of an accompanying shift observed in the resultant inorganic chloride. Hunkeler et al. (*16*) measured the δ^{13}C values of tetrachloroethene (PCE), TCE, *c*-DCE, VC, and ethene across a small plume at a site that was contaminated with PCE. The existence of variable proportions of PCE and the byproducts of reductive dechlorination suggested that intrinsic biodegradation of PCE was occurring at the site. To examine the carbon isotope fractionation effects for the reductive dechlorination process, they measured the δ^{13}C values of chlorinated ethenes during reductive dechlorination of PCE in microcosms prepared with soils from the site. The isotopic shifts they observed in this experiment were consistent with the δ^{13}C data for chlorinated ethenes in groundwater samples collected from the site, leading them to conclude that reductive dechlorination was occurring at the site. In a later study, Sherwood Lollar et al. (*18*) measured the δ^{13}C values of PCE and TCE at a site that was contaminated with both compounds. *c*-DCE, VC, and ethene were also present in the plume, suggesting that reductive dechlorination was occurring at the site. They observed increases in the δ^{13}C values of both the PCE and TCE in the down-gradient portions of the plume (they did not measure the δ^{13}C values of the other ethenes) and concluded that this was due to reductive dechlorination of the PCE and TCE.

In this study, we present the first example of detailed, time-series isotope monitoring of a dynamic system undergoing rapid changes during an enhanced bioremediation field study. At this site, addition of lactate to the source area greatly accelerated the natural process of reductive dechlorination that was already occurring. The concentrations of ethenes in the monitoring wells fluctuated greatly during the experiment, making it impossible to use the concentration data to determine the extent of dechlorination in the system. The isotope data, however, clearly show that all of the TCE that was degraded was fully dechlorinated to ethene.

Materials and Methods

Field Site. A field pilot study was undertaken during 1999 to evaluate the potential for enhanced in situ bioremediation to treat groundwater contaminated with TCE at the Idaho National Engineering and Environmental Laboratory (INEEL) Test Area North (TAN) site (*30*). Between 1955 and 1972, a

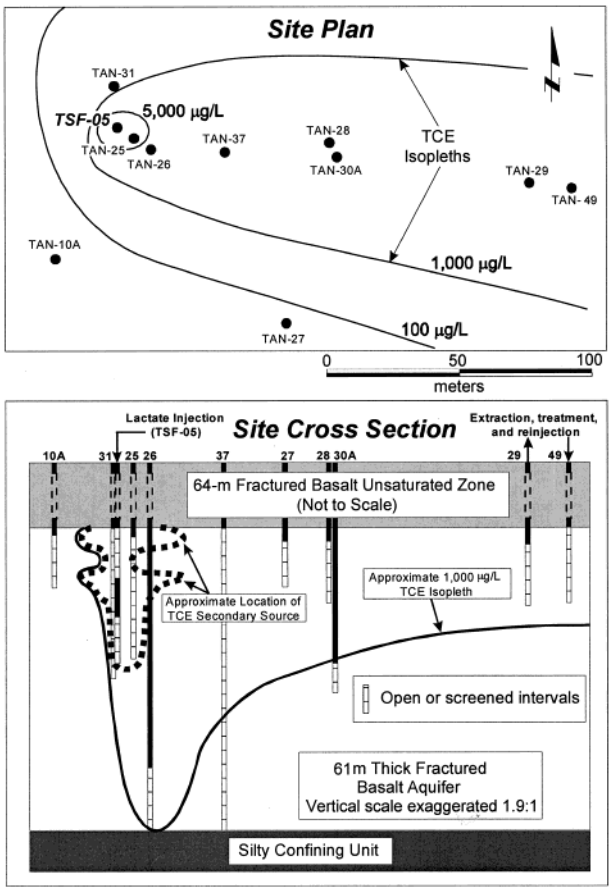

FIGURE 1. Site plan (a) and cross-section (b) of the TCE contaminated site and enhanced bioremediation treatment cell at Test Area North (TAN).

mixture of waste materials, including low-level radioactive isotopes, sewage, and chlorinated solvents were injected into the aquifer through a 95 m deep well (TSF-05 in Figure 1). The result of this activity is a plume of TCE (defined by concentrations greater than 0.04 μM or 5 ppb) with concentrations as high as 2.3 mM (300 ppm) that extends for more than 2 km down gradient from TSF-05. Lesser amounts of *c*-DCE and *t*-DCE were also observed prior to the pilot study, suggesting either that DCE was present in the mixture of solvents in the source area or that some degree of intrinsic reductive dechlorination of TCE occurred prior to this project. The geology of the site consists of permeable basalts with interlayered, relatively impermeable sedimentary layers. The aquifer consists of a 64 m thick unsaturated zone overlying a 61 m thick saturated zone (both consisting of fractured basalt), bounded beneath by a sedimentary interbed.

The pilot study began with a tracer test which involved the injection of 76 L/min of clean water into TSF-05 between Nov 16, 1998 and Dec 11, 1998 (days 0−25). Lactate was introduced into the aquifer through TSF-05 via pulsed injections of 907 kg of sodium lactate dissolved in water for 35 weeks beginning on Jan 7, 1999 and ending on Sept 8, 1999. Although the amount of sodium lactate was kept constant throughout the experiment, the volume of water varied significantly. For the first 3 weeks of injection (beginning on day 52), the sodium lactate was dissolved in 1140 L of water with an injection flow rate of 38 L/min. For the next 4 weeks (beginning on day 78), the total volume injected was increased to 2270 L and the injection flow rate increased to 76 L/min. Over the next 14 weeks (beginning on day 106), the volume was increased 5-fold to 11 400 L with an injection rate of 95 L/min (for the first 7 weeks, it was injected in 2 aliquots/week). Finally, for the last 14 weeks

TABLE 1. $\delta^{13}C$ Measured for Standard Compounds Using Offline Combustion Dual-Inlet Isotope Ratio Mass Spectrometry and Continuous-Flow Isotope Ratio Mass Spectrometry (GC−C−IRMS) with Headspace and Purge-and-Trap Sampling Techniques[a]

	analytical method		
	dual-inlet	headspace GC−C−IRMS	purge-and-trap GC−C−IRMS
compd	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)
TCE	-29.5 ± 0.1 ($n = 3$)	-29.2 ± 0.5 ($n = 22$)	-29.5 ± 0.3 ($n = 14$)
c-DCE	-10.9 ± 0.1 ($n = 2$)	-10.8 ± 0.4 ($n = 6$)	-11.0 ± 0.3 ($n = 9$)
t-DCE		-22.4 ± 0.2 ($n = 4$)	-23.1 ± 0.0 ($n = 2$)
VC		-25.5 ($n = 1$)	-25.0 ± 0.3 ($n = 2$)
ethene		-30.5 ± 0.3 ($n = 5$)	-30.9 ± 0.7 ($n = 5$)

[a] Values represent the means ± standard deviations for *n* samples.

(from day 204), the amount of liquid injected was increased to 22 700 L with a flow rate of 95 L/min. While the increased water volume did not affect the total amount of lactate injected, it did significantly alter the rate at which the lactate spread though the aquifer. After Sept 8 (day 296), lactate injections were stopped until Feb 8, 2000 (day 449), when they were resumed. Throughout the pilot study (beginning with the tracer test), 190 L/min of groundwater was extracted from well TAN-29, treated to remove volatiles, and reinjected in well 49 for hydrologic control. As part of an extensive monitoring program for the study, stable carbon isotope ratios of the chlorinated organics were measured in groundwater samples to evaluate the utility of this technique for assessing treatment progress. Samples for isotope analysis were taken monthly from 10 wells at the site (Figure 1). The data from four of those wells (TAN-25, TAN-26, TAN-29, and TAN-31) were chosen to illustrate significant and representative results. Additional data about the TAN site and the general results of the pilot study are presented in detail elsewhere (*30*, *31*).

Chemical and Isotopic Analyses. Groundwater samples for quantification of ethenes and organic acids were collected throughout the study. Sampling wells were purged prior to sampling using a low-flow technique to minimize purge water volume. Samples were collected from each monitoring well using dedicated submersible pumps and Teflon-lined polyethylene tubing. Details of the well sampling methods are described elsewhere (*30*). Quantification of chlorinated ethene concentrations in field samples was performed at INEEL by solid-phase microextraction of headspace samples using a 75 μm carboxen-poly(dimethylsiloxane) fiber (Supelco, Inc., Bellafonte, PA) and a gas chromatograph with a flame ionization detector (*30*). Lactate analyses were performed by ion exclusion chromatography on a Dionex 4500I with conductivity detection, while acetate, propionate, and butyrate analyses were performed on an HP 5890 series II gas chromatograph with flame ionization detection (*30*).

For isotopic analysis, samples were captured in 40 mL amber glass vials with no headspace, preserved by acidifying the sample to pH less than 2, packed on ice, shipped via overnight delivery, and stored at 4 °C. All isotopic samples were analyzed within 7 days of sampling.

Relatively low concentrations (<1 ppm or 7.6 μM) of chlorinated solvents in groundwater samples required preconcentration prior to isotopic analysis using two different purge-and-trap techniques, both employing a TEKMAR purge-and-trap unit and automatic sampler. The less volatile chloroethenes, TCE, *c*-DCE, and *t*-DCE, were extracted from the samples by purging 0.5−20 mL of the groundwater with helium at 440 mL/min for 10 min and trapping the compounds on an absorbent column filled with SP-2100/Chromosorb W AW and Tenax TA (Supelco Trap G, Supelco, Inc.) at room temperature. After heating the trap to 180 °C to desorb the compounds, they were then further preconcentrated using cryogenic focusing, in which the chloroethenes were flushed from the trap in a helium gas stream and frozen with liquid nitrogen into a stainless steel loop attached to a six-port valve. The valve was then activated, the liquid nitrogen removed, and the sample loop heated with a heat gun in order to inject the sample into the gas chromatograph−combustion−isotope ratio mass spectrometry system (GC−C−IRMS). Vinyl chloride and ethene were extracted by purging 0.5−15 mL of groundwater at 55 mL/min for 10 min through a magnesium perchlorate trap (to remove water vapor) directly into the cryogenic trap before injection into the GC−C−IRMS. These preconcentration methods enabled reproducible isotopic measurement of chloroethene concentrations down to 0.04 μM for TCE to 0.18 μM for ethene (5 ppb).

The GC−C−IRMS system consists of a Hewlett-Packard gas chromatograph, a Micromass combustion interface operated at 850 °C, and a Micromass Isoprime isotope ratio mass spectrometer (Micromass, Manchester, U.K.). A 0.32 mm i.d. Supelco SUPEL-Q-PLOT capillary column was used to separate CO_2, ethene, ethane, VC, *t*-DCE, *c*-DCE, TCE, and PCE. Isotopic compositions are reported in per mil (‰) units from a reference standard using conventional δ notation

$$\delta^{13}C = ((R_{sample}/R_{standard}) - 1) \times 1000 \quad (1)$$

where $R = {}^{13}C/{}^{12}C$. The reference CO_2 gas standard for the GC−C−IRMS was calibrated to Vienna Pee Dee Belemnite (V−PDB) using a dual-inlet mass spectrometer (VG Prism Series II).

TCE (99%; Fisher Scientific, Pittsburgh, PA), *c*-DCE (Supelco, Inc.), *t*-DCE (Supelco, Inc.), VC (Matheson Gas Products, Montgomeryville, PA), ethene, and ethane (Matheson Gas Products) were used for analytical standards. During monthly sampling, a standard TCE solution was analyzed to ensure the calibration of the system. Reference standards were analyzed using both purge-and-trap techniques and direct headspace injection in order to test fractionation by the preconcentration method. In addition, TCE and *c*-DCE were also analyzed by offline combustion and dual-inlet mass spectrometry using a PRISM IRMS for comparison to the online system (Table 1). The standard values exhibit good agreement across analytical methods. The $\delta^{13}C$ values measured for headspace samples were slightly higher than purge-and-trap samples, which concurs with the results of a previous study that showed vaporization of TCE to result in only a slight enrichment of $\delta^{13}C$ (*21*). The purge-and-trap/cryogenic preconcentration method presented here to analyze the isotopic composition of chloroethenes takes up to 30 min/sample but is an effective way to analyze for the low concentrations of TCE and its byproducts necessary for field studies.

Results

Of the wells routinely monitored during the pilot study, TAN-29 is situated furthest down gradient from well TSF-05, which

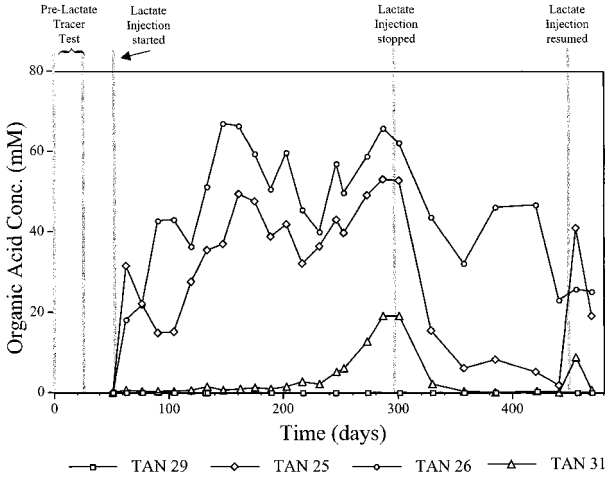

FIGURE 2. Organic acid concentrations at TAN-29 a shallow well located outside the zone of lactate exposure, TAN-25 a shallow well directly down gradient from the lactate injection well, TAN-26 a deep well located down gradient from the lactate injection well, and TAN-31 a shallow well located peripheral to the lactate injection well.

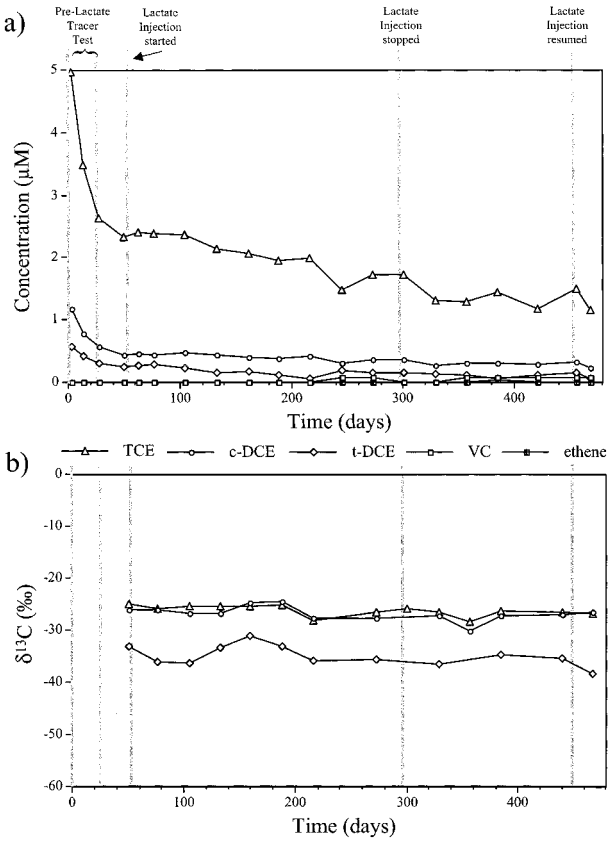

FIGURE 3. Solvent concentration (a) and isotope data (b) at well TAN-29 located outside the zone of lactate exposure.

was both the source of contamination and the lactate injection point (Figure 1). Organic acid concentrations (Figure 2) and geochemical results (COD, DO, pH, specific conductivity measurements (*30*)) indicate that TAN-29 was outside the influence of lactate injection throughout the study, so data from this well represent a negative control for enhanced bioremediation. During the experiment, water was pumped from TAN-29, treated to remove the chlorinated solvents, and injected into TAN-49 (~8 m down gradient from TAN-29). In response to this pump-and-treat operation, the concentrations of TCE, *c*-DCE, and *t*-DCE decreased with

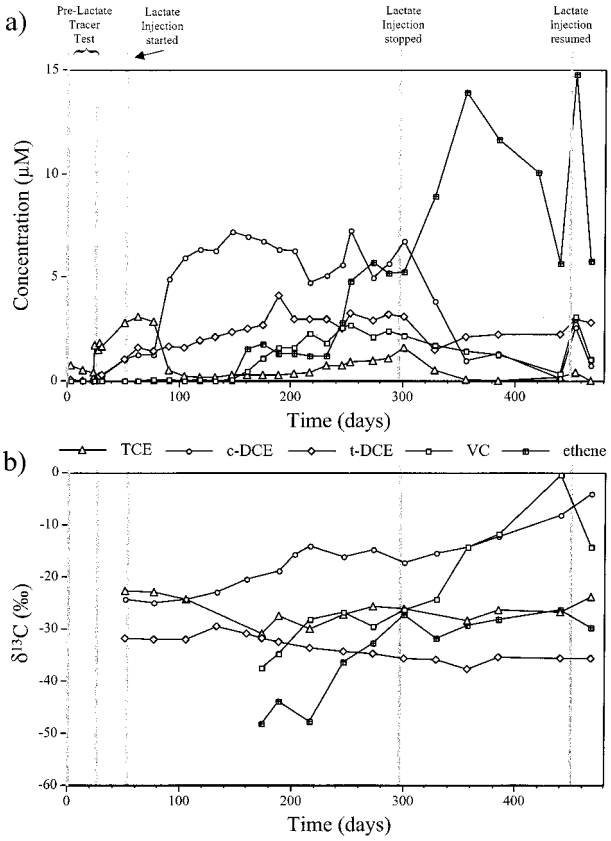

FIGURE 4. Solvent concentration (a) and isotope data (b) at down gradient shallow well TAN-25 located within the zone of high lactate exposure.

time (Figure 3). The $\delta^{13}C$ values of TCE and *c*-DCE were relatively similar during the study. TCE ranged from −25.0‰ to −28.5‰ and averaged −26.3‰, while *c*-DCE ranged from −24.4‰ to −30.3‰ and averaged −26.8‰. The $\delta^{13}C$ values of *t*-DCE were consistently lower, ranging from −31.2‰ to −38.3 ‰ and averaging −35.0‰. Although there was some variability in the isotope data, there were no systematic trends. The data scatter is believed to be characteristic of the general variability in the plume and to be typical of the range of values that would be expected in a system with no bioremediation. Similar results were obtained for four additional monitoring wells that were also outside of the zone of lactate exposure (wells TAN-28, 30A, 27, and 10A).

At TAN-25, the monitoring well closest to the lactate injection well TSF-05, samples were collected from 67 m depth, just below the water table. As expected, high concentrations of organic acids were observed at this well throughout the lactate injection period (10−60 mM); and organic acids persisted at this well after lactate injection ceased (Figure 2). Because lactate was quickly converted to acetate, propionate, and butyrate in this study, total organic acid concentrations provide more information with respect to the spread of electron donor than lactate concentrations alone would.

The chloroethene concentrations and isotope ratios for TAN-25 are plotted in Figure 4. The effects of the injection of clean water during the pre-lactate tracer test are apparent in the observable decline in TCE, *c*-DCE, and *t*-DCE concentration data in the first 26 days (Figure 4a). After the tracer test was concluded, the concentrations of all three compounds rebounded before lactate injection began and remained relatively constant during the first month of lactate injection. During that time, the $\delta^{13}C$ values of those compounds were in the same range as at TAN-29 (TCE was ~3‰

higher in TAN-25). After a month of lactate injection, the concentration of TCE began to decline and the concentration of *c*-DCE began to increase, indicating that reductive dechlorination was beginning in the vicinity of TAN-25. This continued for 2 months, during which time the $\delta^{13}C$ value of *c*-DCE began to rise (~days 90−150). This isotopic rise is significant, because it indicates that the *c*-DCE is being further degraded to VC even before any VC or ethene are detected in the samples.

The increases in volume and flow rate of water injected with the lactate after day 106 and again at day 204 appear to have caused increased mobilization of TCE from the source area. The concentration of TCE began to rise on day 133 and the $\delta^{13}C$ value of TCE dropped to less than −30‰ (probably approaching the isotope composition of undegraded TCE in the source area). The concentration of *t*-DCE also began to increase while the $\delta^{13}C$ values of *t*-DCE decreased, suggesting that *t*-DCE may also have been mobilized from the source area. VC and ethene began to appear at TAN-25 on day 160. The initial $\delta^{13}C$ values of these compounds were very low (−37.4‰ for VC and −48.1‰ for ethene), reflecting the large kinetic fractionation effect caused by reductive dechlorination of DCE to VC and VC to ethene. With time, the $\delta^{13}C$ values of *c*-DCE, VC, and ethene all increased, indicating greater and greater degrees of reductive dechlorination.

On day 296, lactate injection was temporarily stopped, and no water was pumped into TSF-05. This resulted in a rapid decline in the concentrations of TCE, *c*-DCE, and VC. The $\delta^{13}C$ values of *c*-DCE and VC increased to very high values (−8.2‰ for *c*-DCE and −0.4‰ for VC), indicating substantial reductive dechlorination. Notably, the concentration of ethene increased during this time period to as much as 4 times the initial concentration of TCE in this well, but the $\delta^{13}C$ value of the ethene remained relatively constant at about −29‰ (approximately equal to the $\delta^{13}C$ value of TCE from the source area). Clearly, the high ethene concentrations were primarily caused by transport from other areas where complete reductive dechlorination of dissolved TCE was occurring. In addition, the concentrations and $\delta^{13}C$ values of *t*-DCE remained relatively constant during this period, suggesting that little, if any, reductive dechlorination of *t*-DCE was occurring. Finally, on day 449, lactate injection was resumed, and the concentrations of TCE, *c*-DCE, and VC all increased significantly as they were once again flushed out of the source area.

TAN-26 is located ~5 m down gradient from TAN-25 but was sampled close to the base of the aquifer at a depth of 119 m (Figure 1). High concentrations of organic acids (20−70 mM) were observed at this well throughout the lactate injection period and after lactate injection ceased (Figure 2). The concentrations and carbon isotope ratios measured for samples from TAN-26 are plotted in Figure 5. The effects of the pre-lactate injection tracer test were minimal in this well. After lactate injection began, however, the concentrations of TCE, *c*-DCE, and *t*-DCE increased by more than an order of magnitude. The $\delta^{13}C$ values of TCE and *c*-DCE (no analyses of *t*-DCE for these time points are available) were in the same general range as observed in TAN-29, suggesting that the increase in concentration resulted from mass transport. It appears that the injection of the dense lactate solution (60% sodium lactate in water) facilitated the transport of the contaminants deeper into the aquifer (*30*, *32*).

About 6 weeks after lactate injection began, the concentration of TCE began to decrease and the concentration of *c*-DCE began to rise, indicating that enhanced reductive dechlorination had begun in the vicinity of this well (2 weeks after it started in TAN-25). By day 146, the TCE was gone and *c*-DCE was approaching its peak concentration. However, significant amounts of VC and ethene were not observed until a month later. The $\delta^{13}C$ values of the *c*-DCE were

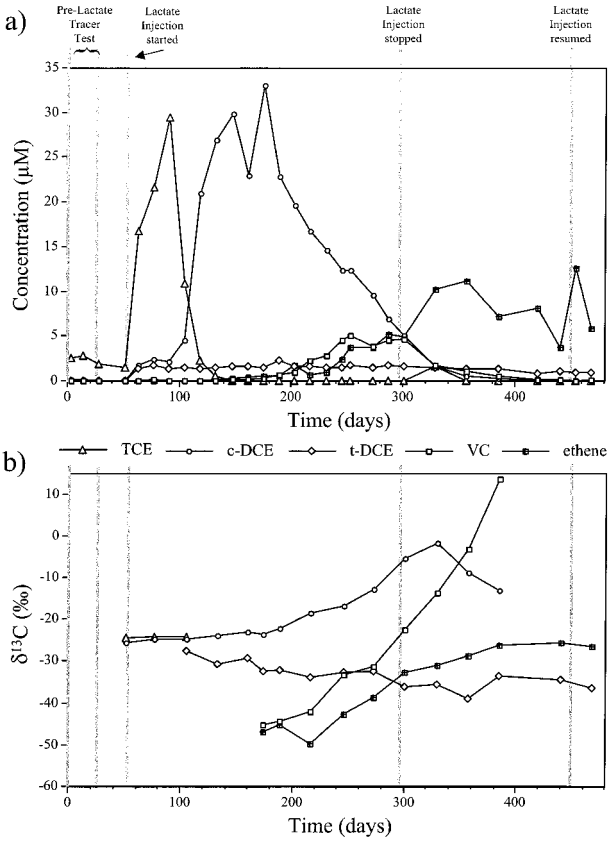

FIGURE 5. Solvent concentration (a) and isotope data (b) at down gradient deep well TAN-26 located within the zone of high lactate exposure.

relatively constant until that time, suggesting that the reductive dechlorination process was not proceeding beyond *c*-DCE. After that point, the concentrations of *c*-DCE dropped continuously as the concentrations of VC and ethene increased. At the same time, the $\delta^{13}C$ values of all three compounds increased steadily, indicating that reductive dechlorination was progressing to ethene. This process went to completion after lactate injection stopped with the disappearance of *c*-DCE and VC and build-up of ethene with $\delta^{13}C$ values of around −26‰.

For the entire time that lactate was being injected, the concentration of *t*-DCE at TAN-26 was relatively constant (ranging between 1.3 and 2.2 μM). Only after lactate injection stopped did the concentration begin to decrease slowly (to a low of 0.7 μM). The $\delta^{13}C$ values of *t*-DCE started out above −30‰ and dropped to less than −35‰ after lactate injection stopped. This suggests that if reductive dechlorination of *t*-DCE occurred at all, it was very slow and transport from the source area was the primary process affecting the concentration and $\delta^{13}C$ values of *t*-DCE.

TAN-31 is located approximately 17 m across the hydraulic gradient from TSF-05 (Figure 1). Low concentrations of organic acids (<20 mM) were observed at this well during the lactate injection period with concentrations increasing after injection volumes increased on day 206 and dropping back down after lactate injection ceased (Figure 2). The concentration and isotopic data collected for TAN-31 are shown in Figure 6. The concentration of TCE dropped slightly during the pre-lactate tracer test but rebounded (along with *c*-DCE and *t*-DCE) before lactate injection started. After lactate injection began, the concentrations of the chloroethenes remained fairly constant for 6 weeks. After the injection volumes were increased on day 106, the concentrations of TCE began to drop and of *c*-DCE increased indicating that

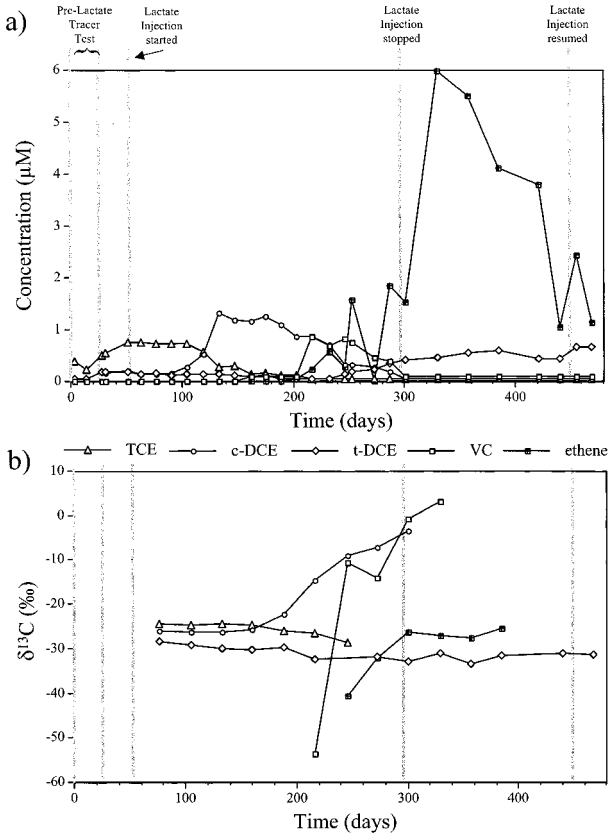

FIGURE 6. Solvent concentration (a) and isotope data (b) at peripheral well TAN-31 which was located within the zone of limited lactate exposure.

the initial step of the reductive dechlorination process had begun. There was, however, no significant VC or ethene production until after day 200. Prior to day 160, the $\delta^{13}C$ values of TCE and *c*-DCE were relatively constant, averaging −24.6‰ and −26.2‰, respectively. After that, the $\delta^{13}C$ values of TCE started to drop slightly (to −28.6‰), indicating an influx of TCE from the source area. At the same time, the $\delta^{13}C$ values of *c*-DCE began increasing, signifying that reductive dechlorination of *c*-DCE had begun (approximately 1 month before any VC was detected in the well). After day 206, when the organic acid concentrations in the well began to increase rapidly, the concentrations of *c*-DCE dropped steadily and the $\delta^{13}C$ value increased to −3.4‰. Concurrently, the concentration of VC jumped up to almost 1 μM and then began dropping. The $\delta^{13}C$ values of the VC started out very low (−53.6‰) and rose rapidly, indicating substantial dechlorination to ethene. Ethene concentrations increased, and the $\delta^{13}C$ value of the ethene reached −26.6‰, a value similar to that of the TCE. In addition, the *t*-DCE increased to higher concentrations than were observed before the test began and the $\delta^{13}C$ values decreased to around −33‰, suggesting transport from the source area. After lactate injection ended on day 296, the organic acid concentrations decreased, and the concentration of ethene jumped to almost an order of magnitude higher than the initial concentration of TCE in this well while the $\delta^{13}C$ values of ethene remained relatively constant (averaging −26.7‰).

Discussion

The $\delta^{13}C$ data presented in this paper demonstrate how isotope measurements can be used to distinguish between concentration changes that occur due to physical processes such as groundwater transport from those that occur due to bioremediation. During the early stages of the bioremediation experiment at the TAN site, the $\delta^{13}C$ values of TCE, *c*-DCE, and *t*-DCE stayed relatively constant, despite large fluctuations in the concentrations of all three compounds caused by the injection of water. This is most apparent in the TAN-29 data, where the $\delta^{13}C$ values were unaffected despite continuous pumping, treating, and reinjection of groundwater from this well.

The isotope data also indicate that some intrinsic degradation of TCE occurred prior to the pilot study. The initial $\delta^{13}C$ values of TCE were somewhat higher than would be expected for TCE that had not been fractionated by degradation, especially in the TAN-25 well. The $\delta^{13}C$ values of commercially produced TCE that have been published in the literature (*20*, *33*) and measured for this study have been between −27‰ and −32‰. The fractionation effects observed for the transformation of TCE to *c*-DCE are generally small (<5‰), but if there is extensive degradation of TCE (>25%), it is possible that the $\delta^{13}C$ values of the residual TCE can be shifted to significantly higher values, such as was observed by Lollar et al. (*18*) at Dover Air Force Base.

The shift in the $\delta^{13}C$ values of TCE away from the source area is also helpful for identifying the influx of freshly dissolved TCE in downstream wells. Free-phase DNAPL has been found in the vicinity of TSF-05. This TCE would not have been affected by reductive dechlorination and should have $\delta^{13}C$ values representative of the TCE injected into TSF-05. The clearest example of this is in TAN-25, where the concentrations of TCE begin increasing after day 130 and the $\delta^{13}C$ values drop to −31‰, indicating that the lactate injections were causing some of the free-phase TCE around TSF-05 to be dissolved into the groundwater and transported down gradient.

The most useful aspect of stable isotope monitoring of chlorinated solvents at the TAN site was the ability to demonstrate that complete transformation of dissolved TCE to ethene could be achieved by enhanced bioremediation using lactate as an electron donor. Isotope data from all wells within the zone of lactate influence exhibited large kinetic isotope effects accompanying the degradation of *c*-DCE and VC. The $\delta^{13}C$ values of both VC and ethene were initially very low but increased rapidly as degradation progressed. Eventually, the $\delta^{13}C$ values of ethene in all of the wells subject to organic acid exposure reached the $\delta^{13}C$ of the original dissolved TCE, confirming that complete reductive dechlorination was occurring. If incomplete transformation of TCE had occurred, leading to the accumulation of *c*-DCE or VC, then the $\delta^{13}C$ value of the final product would have remained lower than that of the TCE (i.e., $\delta^{13}C_{ethene} < \delta^{13}C_{TCE}$) due to the large kinetic isotopic effects. Given the extreme toxicity and volatility of VC, it is critical that its complete conversion to ethene be demonstrated during a successful enhanced reductive dechlorination process. At this site, a mass balance approach based on concentration data would not have been effective for demonstrating that complete transformation of dissolved TCE had occurred due to the confounding effects of transport through the fractured rock aquifer. In addition, background chloride concentrations were in the range of 1−4 mM, precluding the use of chloride measurements for tracking solvent transformations in the micromolar range.

In this study, the isotope measurements also indicated that there was little or no degradation of *t*-DCE caused by the lactate injections. The source of the *t*-DCE in the plume was not clear. It could have been produced during either biologic or abiotic reductive dechlorination of TCE but is usually generated in much smaller proportions than *c*-DCE (*34*). It is also possible that it was a co-contaminant with the injected TCE. Although not widespread, *t*-DCE was used for industrial applications (primarily as a solvent for waxes or resins), and one technique for manufacturing TCE involves chlorination of DCE (either the trans or cis isomers). At any

rate, *t*-DCE was clearly present in the source area of the TAN plume. The $\delta^{13}C$ values measured for *t*-DCE tended to decrease throughout the experiment (especially in TAN-25 and TAN-26, the two wells nearest to TSF-05), suggesting possibly that the initial value of *t*-DCE was less than −35‰ and that some intrinsic degradation of the *t*-DCE may have previously occurred in the plume (following the same general arguments used for TCE). However, the concentrations of *t*-DCE did not decrease and the $\delta^{13}C$ values did not increase in wells where enhanced reductive dechlorination of the other chlorinated solvents was occurring. In fact, in TAN-31, the concentration of *t*-DCE increased concomitant with the increase in organic acids around day 240 and remained elevated for the duration of the study (Figure 6), supporting the conclusion that *t*-DCE was mobilized from the source area by the lactate injection but not degraded by the enhanced biological activity.

The time-series isotopic results of this study are consistent with the single-time period results from previous field studies conducted on sites undergoing intrinsic bioremediation of chlorinated ethenes without electron-donor enhancement (*16*, *18*), in support of the conclusion that stable carbon isotopic monitoring of chloroethenes can provide direct evidence for the extent of biological reductive dechlorination of TCE. In addition, the results of this study show that a time-series of isotopic measurements taken from individual wells, rather than the more typical approach of monitoring along a transect of wells during a single time period, can be extremely useful for tracking the progress of a bioremediation enhancement strategy. In fact, the time-series monitoring of reductive dechlorination observed at TAN yielded results very similar to those reported in microcosm experiments (*16*, *17*, *26*, *32*) and verifies that the kinetic isotopic fractionation observed in laboratory studies can indeed occur during a biological enhancement process in the field.

The ultimate goal for this work is to integrate isotope ratios into a coupled reaction-transport model that could be used to monitor in situ reductive dechlorination of TCE (or PCE) and quantify the total amount of contaminant that has been degraded. The results of this pilot study clearly demonstrate the sensitivity of carbon isotope measurements to the processes that occurred during the enhancement experiment. Shifts in the $\delta^{13}C$ values of intermediary products of reductive dechlorination often preceded detection of the next compound in the chain. For example, the $\delta^{13}C$ values of *c*-DCE began to increase in TAN-25 and TAN-31 before there were measurable amounts of VC. Further, processes that caused large changes in concentration, such as groundwater transport, did not affect the isotope measurements.

Acknowledgments

This field project was funded by NIEHS Grant P42-ES04705 and a graduate fellowship through the UC Toxic Substances Research and Teaching Program. Partial support for this work was also provided by the Assistant Secretary for Environmental Management, Office of Science and Technology, under the Environmental Management Science Program of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. We also thank three anonymous reviewers for their thoughtful comments.

Literature Cited

(1) National Research Council. *Alternatives for Groundwater Cleanup*; National Academy Press: Washington, DC, 1994.
(2) U.S. EPA. EPA Groundwater Issue Paper, EPA/540/4-91-002, 1991.
(3) MacKay, D. M.; Cherry, J. A. *Environ. Sci. Technol.* **1989**, *23*, 630−636.
(4) Bouwer, E. J. *Environmental Microbiology*; Wiley-Liss Inc.: New York, 1992; pp 287−318.
(5) Vogel, T. M.; McCarty, P. L. *Appl. Environ. Microbiol.* **1985**, *49*, 1080−1083.
(6) McCarty, P. L. *Science* **1997**, *276*, 1521−1522.
(7) Graves, R. W.; Hinchee, R. E.; Jensen, T. M.; Graves, A. E.; Wiedemeier, T.; Wheeler, M.; Elliot, R. In *Proc. 4th International In Situ and On-Site Bioremediation Symposium*; Alleman, B. C., Leeson, A., Eds.; Battelle Press: New Orleans, LA, 1997; Vol. 3.
(8) Freedman, D. L.; Gossett, J. M. *Appl. Environ. Microbiol.* **1989**, *55*, 2144−2151.
(9) Sharma, P.; McCarty, P. L. *Appl. Environ. Microbiol.* **1996**, *62*, 761−765.
(10) Maymo-Gatell, X.; Chien, Y.; Gossett, J. M.; Zinder, S. H. *Science* **1997**, *276*, 1568−1571.
(11) Wiedemeier, T. H.; Rifai, H. S.; Newell, C. J.; Wilson, J. T. Natural Attenuation of Fuels and Chlorinated Solvents; John Wiley and Sons Inc.: New York, 1999,
(12) Gossett, J. M. *Environ. Sci. Technol.* **1987**, *21*, 202−208.
(13) Mackay, D.; Shiu, W. Y. *J. Phys. Chem. Ref. Data* **1981**, *10*, 1175−1199.
(14) Veith, G. D.; Call, D. J.; Brooke, L. T. *Can. J. Fish. Aquat. Sci.* **1983**, *40*, 743−748.
(15) Callahan, M. A.; Slimak, M. W.; Gabel, N. W.; May, I. P.; Fowler, C. F.; Freed, J. R.; Jennings, P.; Durfee, R. L.; Gould, C. EPA-440/4-79-029b, 1979.
(16) Hunkeler, D.; Aravena, R.; Butler, B. *Environ. Sci. Technol.* **1999**, *33*, 2733−2738.
(17) Slater, G. F.; Sherwood Lollar, B.; Sleep, B. E.; Edwards, E. A. *Environ. Sci. Technol.* **2001**, *35*, 901−907.
(18) Sherwood Lollar, B.; Slater, G. F.; Sleep, B.; Witt, M.; Klecka, G. M.; Harkness, M.; Spivack, J. *Environ. Sci. Technol.* **2001**, *35*, 261−269.
(19) Galimov, E. M. *The Biological Fractionation of Isotopes*; Academic Press: Orlando, FL, 1985.
(20) Van Warmerdam, E. M.; Frape, S. K.; Aravena, R. J.; Drimmie, R. J.; Flatt, H.; Cherry, J. A. *Appl. Geochem.* **1995**, *10*, 547−552.
(21) Poulson, S. R., Drever, J. *Environ. Sci. Technol.* **1999**, *33*, 3689−3694.
(22) Harrington, R. R.; Poulson, S. R.; Drever, J. I.; Colberg, P. J. S.; Kelly, E. F. *Org. Geochem.* **1999**, *30*, 765−776.
(23) Slater, G. F.; Dempster, H. D.; Sherwood Lollar, B.; Ahad, J. *Environ. Sci. Technol.* **1999**, *33*, 190−194.
(24) Slater, G. F.; Ahad, J. M. E.; Sherwood Lollar, B.; Allen-King, R.; Sleep, B. *Anal. Chem.* **2000**, *72*, 5669−5672.
(25) Huang, L.; Sturchio, N. C.; Abrajano, T., Jr.; Heraty, L. J.; Holt, B. D. *Org. Geochem.* **1999**, *30*, 777−786.
(26) Bloom, Y.; Aravena, R.; Hunkeler, D.; Edwards, E.; Frape, S. K. *Environ. Sci. Technol.* **2000**, *34*, 2768−2772.
(27) Dayan, H.; Abrajano, T.; Sturchio, N. C.; Winsor, L. *Org. Geochem.* **1999**, *30*, 755−763.
(28) Bill, M.; Schuth, C.; Barth, J. A. C.; Kalin, R. M. *Chemosphere* **2001**, *44*, 1281−1286.
(29) Sturchio, N. C.; Clausen, J. L.; Heraty, L. J.; Huang, L.; Holt, B. D.; Abrajano, T. A. *Environ. Sci. Technol.* **1998**, *32*, 3037−3042.
(30) Sorenson, K. S.; Peterson, L. N.; Ely, R. L. Enhanced in situ Reductive Dechlorination of Trichloroethene in a Deep, Basalt Aquifer. *Ground Water*, submitted for publication.
(31) Sorenson, K. S.; Peterson, L. N.; Ely, R. L. In *Engineered Approaches for in situ Bioremediation of Chlorinated Solvent Contamination*; Leeson, A., Alleman, B. C., Eds.; Battelle Press: Columbus, OH, 1999; pp 147−156.
(32) Sorenson, K. S.; Ely, R. L. *Enhanced Bioremediation for Treatment of Chlorinated Solvent Residual Source Areas*; American Chemical Society, Environmental Chemistry Division, Preprints of Extended Abstracts, 2001; 41, pp 1092−1097.
(33) Holt, B. D.; Sturchio, N. C.; Abrajano, T. A.; Heraty, L. J. *Anal. Chem.* **1997**, *69* (14), 2727−2733.
(34) Rittman, B. E.; McCarty, P. L. *Environmental Biotechnology: Principles and Applications*; McGraw-Hill: New York, 2001.

Received for review July 25, 2001. Revised manuscript received February 25, 2002. Accepted February 27, 2002.

ES011162D