

Monitoring Microbial Dechlorination of Tetrachloroethene (PCE) in Groundwater Using Compound-Specific Stable Carbon Isotope Ratios: Microcosm and Field Studies

D. HUNKELER,[†] R. ARAVENA,^{*,†} AND B. J. BUTLER[‡]

Departments of Earth Sciences and Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1 Canada

The determination of compound-specific stable isotope ratios is a promising new tool to assess biodegradation of organic compounds in groundwater. In this study, the occurrence of carbon isotope fractionation during dechlorination of tetrachloroethene (PCE) to ethene was evaluated in a PCE-contaminated aquifer and in a microcosm that was based on aquifer material from the site. In the microcosm, all dechlorination steps were accompanied by carbon isotope fractionation. The largest fractionation occurred during dechlorination of *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC), resulting in a large enrichment of ¹³C in the remaining cDCE and VC. Stable carbon isotope ratios ($\delta^{13}\text{C}$) of cDCE and VC increased from -25.7 to -1.5‰ and -37.0 to -2.5‰ , respectively. The $\delta^{13}\text{C}$ of ethene was initially -60.2‰ and approached the $\delta^{13}\text{C}$ of the added PCE (-27.3‰) as dechlorination came to completion. A similar carbon isotope pattern was observed for PCE dechlorination at the field site. Strong enrichment of ¹³C in cDCE and VC during microbial dechlorination may serve as a powerful tool to monitor the last two dechlorination steps, which frequently determine the rate of complete dechlorination of chlorinated ethenes at field sites undergoing intrinsic bioremediation.

Introduction

Contamination of groundwater with chlorinated solvents is a common environmental problem. As a nonaqueous phase, chlorinated solvents spread in complex patterns in heterogeneous aquifers (1). Furthermore, there are often multiple sources of chlorinated solvents in industrial areas. Highly chlorinated solvents such as tetrachloroethene (PCE) or carbon tetrachloride are persistent or degrade only slowly under aerobic conditions in aquifers, while under reducing conditions partial or complete microbial dechlorination occurs (e.g. refs 2 and 3). Less chlorinated solvents such as trichloroethene (TCE) can also be biodegraded under aerobic conditions (4). The complex spreading pattern of chlorinated solvents, the presence of multiple sources, and the occurrence

of different transformation processes often leads to complex plumes of dissolved chlorinated solvents and various degradation products which are difficult to interpret.

The determination of compound-specific isotope ratios is a promising new tool to gain insight into the origin and fate of chlorinated solvents in aquifers (5). In a previous study, it has been shown that carbon and chlorine isotope ratios differ in chlorinated solvents produced by different manufacturers (5). Compound-specific isotope ratios may be used to (i) distinguish between different sources or events of contamination if isotope ratios are conserved during transport in the subsurface or (ii) demonstrate the occurrence of biodegradation if biodegradation is accompanied by a characteristic isotope fractionation. Both potential applications require knowledge of the isotope fractionation occurring during the physical, chemical, and biological processes that affect chlorinated solvents in the subsurface. In a previous study, it has been shown that, in the absence of biodegradation, the carbon isotope ratio ($\delta^{13}\text{C}$) of PCE is conserved during transport of PCE in an aquifer (6). The major goal of this study was to investigate the occurrence and degree of carbon isotope fractionation during microbial dechlorination of PCE to ethene.

During biologically mediated processes, a shift in the isotope ratios between the precursor and the product can frequently be observed which is denoted as isotope fractionation or more specifically kinetic isotope fractionation (7). As a simplification, the first term is used in this paper. Isotope fractionation occurs since bonds formed by the lighter isotopes of an element usually are weaker, and thus react faster, than bonds formed by the heavy isotopes. This leads to a progressive enrichment of the heavy isotopes in the precursor, while the product becomes depleted in the heavy isotopes. The occurrence of isotope fractionation was successfully used to demonstrate denitrification (8, 9) and sulfate reduction (10, 11) in aquifers. In addition, stable carbon isotopes have proved to be a useful tool to distinguish between different sources of DIC in uncontaminated (12) and contaminated aquifers (13–16). However, little is known yet about the influence of microbial degradation on isotope ratios in individual organic contaminants. No significant carbon isotope fractionation was observed during degradation of aromatic (17) and polyaromatic (18) hydrocarbons. In contrast, a large carbon isotope fractionation occurs during production (19) and oxidation (20) of methane in aquifers. In a recent study, a small chloride isotope fractionation during oxidation of TCE has been reported (21). To our knowledge, no data have been reported to date on carbon isotope fractionation during complete dechlorination of chlorinated ethenes.

In many laboratory studies, it has been shown that anaerobic bacteria can dechlorinate PCE (3, 22–30). Dechlorination of PCE usually occurs sequentially leading to the formation of TCE, *cis*-1,2-dichloroethene (cDCE), and vinyl chloride (VC) as intermediate products. VC is often the slowest to dechlorinate and frequently accumulates. In most cases, ethene is the end product of dechlorination, although in some studies formation of ethane (27) and CO₂ (31) has been observed. Each dechlorination step requires two electrons, and therefore an electron donor has to be present. A number of organic compounds have been successfully used to stimulate microbial dechlorination of PCE to ethene including methanol, acetate, butyrate, lactate, propionate, and H₂. Recent studies suggest that H₂ is frequently the direct electron donor used for dechlorination while organic substrates indirectly supply H₂ via fermentation (3). Dechlorination of

* Corresponding author phone: (519)885-1211; fax: (519)746-7484; e-mail: roaraven@sciborg.uwaterloo.ca.

[†] Department of Earth Sciences.

[‡] Department of Biology.

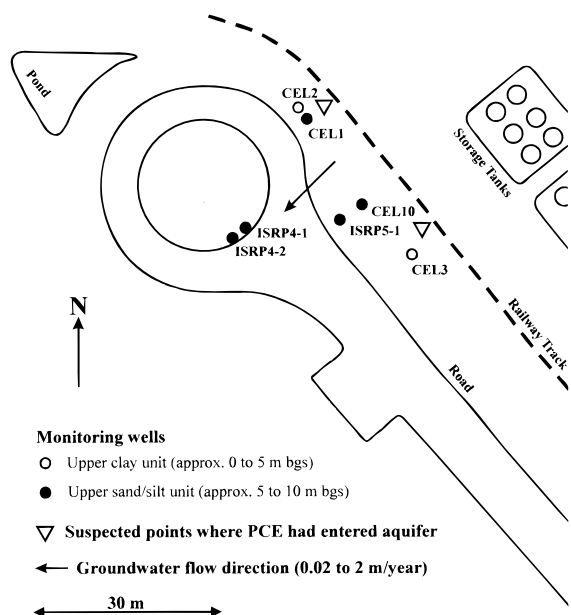


FIGURE 1. Plan of the field site at Toronto, Ontario, Canada.

PCE under anaerobic conditions had been observed at several field sites (e.g. ref 24).

In this study, the occurrence and degree of carbon isotope fractionation during microbial dechlorination of PCE to ethene is evaluated in a laboratory experiment (microcosm) and at a field site. The study site is a PCE-contaminated aquifer located in Toronto, Canada. The microcosm experiment was performed using aquifer sediments and groundwater from the field site. The results of the microcosm and field studies are compared, and conclusions for the application of stable carbon isotopes to assess the origin and fate of chlorinated solvents are drawn.

Material and Methods

Field Site. The field site is located at a bulk-chemical transfer facility in Toronto, Ontario, Canada. The field site is described in detail in Major et al. (24). The subsurface is composed of clay till (upper clay) underlain by a sand/silt unit (upper sand/silt), a clay unit (lower clay), a layered sand/silt unit (lower sand/silt), and bedrock. The upper clay unit is weathered and fractured, while the lower clay seems to be a competent aquitard. Generally, groundwater flow is from NE to SW (Figure 1) with a horizontal velocity of 0.02–2 m/year.

The previous and current owners of the facility stored and transferred similar organic solvents (methanol, methyl ethyl ketone, vinyl and ethyl acetate, and butyl acrylate) with one exception. About 18 years ago PCE was stored at the site. Site investigations revealed that small releases of PCE occurred during coupling and uncoupling of railway tanker cars. The spilled PCE probably migrated as nonaqueous phase along the spur line gravel pack and found at least two entry points into the aquifer: one between CEL3 and CEL10 and the other close to CEL1 (Figure 1). PCE as nonaqueous phase was detected at CEL3. Dissolved PCE was found in the upper clay and the upper sand/silt unit. However, spreading of the PCE was greater in the sand/silt unit due to a higher groundwater flow velocity in this unit. In addition to dissolved PCE, groundwater samples contained significant concentrations of methanol, acetate, TCE, cDCE, VC, and ethene. This indicated that dechlorination was occurring at the site, probably stimulated by methanol or acetate that had been spilled at another occasion. Additional evidence of microbial dechlorination of PCE at the site was provided by microcosm

TABLE 1. Location of Screens of Monitoring Wells at Toronto Site

monitoring well	casing elevation (masl) ^a	screened interval (mbgs) ^b
Upper Clay Unit		
CEL2	161.25	2.0–4.5
CEL3	161.82	1.8–4.3
Upper Sand/Silt Unit		
CEL1	161.23	7.6–9.1
CEL10	161.18	5.8–7.2
ISRP 4-1	161.81	7.7–9.1
ISRP 4-2	161.89	5.5–6.9
ISRP 5-1	161.92	7.6–9.0

^a Abbreviation: masl, meters above sea level. ^b Abbreviation: mbgs, meters below ground surface.

experiments using site aquifer sediments and groundwater (24). PCE dechlorination was observed in microcosms amended with methanol and/or acetate but not in control microcosms with autoclaved site sediment and HgCl₂. An in situ microcosm study also confirmed PCE dechlorination at the site (32). In most of the monitoring wells in the upper silt/sand unit CH₄ was found, and in several monitoring wells SO₄²⁻ was absent or concentrations were low compared to the background, which indicates that this unit was under sulfate-reducing and methanogenic conditions.

Groundwater Sampling. Groundwater samples were taken on August 28, 1998. Sampling was performed after purging the wells by removing three well volumes of water. Groundwater samples were only taken in monitoring wells (Table 1) in which total concentrations of chlorinated ethenes had been >10 μM in previous sampling campaigns. As a simplification, the term chlorinated ethenes includes ethene. For analysis of concentrations of chlorinated ethenes, ethane, and methane, 40 mL vials were filled without headspace and sealed with Teflon lined caps; for stable carbon isotope ratio measurements 125 mL bottles were similarly filled and sealed. The samples were preserved by adding Na-azide.

Preparation of Microcosm. The microcosm used for the carbon isotope study is based on microcosms from an earlier study to confirm PCE dechlorination at the site (24). The sampling of aquifer material and the setup of the initial microcosms are described in detail in ref 24. For this study, a 250 mL microcosm was prepared in an anaerobic glovebox using 6 g wet weight of aquifer material from the initial microcosms. This material had been three times amended with methanol and PCE since it had been sampled. Two hundred milliliters of site groundwater taken from monitoring well CEL10 were added to the microcosm bottle, which was then sealed with a screw-cap Mininert valve (Vici Precision Sampling, Baton Rouge, LA, U.S.). Before addition to the microcosm, the groundwater was purged for 24 h with sterile prepurified N₂ to remove chlorinated ethenes, ethane, and methane. The microcosm was amended with methanol (1.6 mM or 50 ppm) and PCE (85 μM or 14 ppm).

Chemical Analysis. For field samples, concentrations of chlorinated ethenes were determined using a Hewlett-Packard (Hewlett-Packard, Palo Alto, CA, U.S.) gas chromatograph (GC) equipped with a 30 m DB-VRX column (J&W Scientific, Folsom, CA, U.S.), a photoionization detector, and a Varian headspace autosampler (Varian, Walnut Creek, CA, U.S.). With this setup, it was possible to separate VC, 1,1-dichloroethene (11DCE), *trans*-1,2-dichloroethene (tDCE), cDCE, TCE, and PCE. Concentrations of dissolved hydrocarbons (methane, ethane, and ethene) were measured using a Hewlett-Packard GC equipped with a 30 m Megabore GS-Q column (J&W Scientific) and a flame ionization detector (24). The standard uncertainty of the concentration measurements was ≤5%; the limit of quantification was 0.2 μM.

For the microcosm, headspace concentrations of chlorinated ethenes, and ethane were determined using the GC-C-IRMS instrument described below. Concentrations of dissolved compounds were calculated from concentrations in the headspace using Henry's law. To allow for comparison of production and consumption of the different compounds, the sum of the amount of each compound in the gas and aqueous phase of the microcosm is reported. The standard uncertainty of the sum of each compound is 10%.

Stable Carbon Isotope Analysis. Compound-specific isotope ratios were determined in the Environmental Isotope Laboratory (EIL) of the University of Waterloo using a gas chromatograph combustion isotope-ratio mass spectrometry (GC-C-IRMS) system. The GC-C-IRMS system consisted of a Hewlett-Packard GC with a split/splitless injector, a Micromass combustion interface operated at 850 °C, and a Micromass Isochrom isotope-ratio mass spectrometer (Micromass, Manchester, U.K.). The GC was equipped with a GS-GasPro column (J&W Scientific), which is capable of separating the following compounds: CH₄, CO₂, ethane, ethene, VC, 11DCE, tDCE, cDCE, TCE, and PCE. The minimal amount of carbon required to enter the GC column in order to be able to reproducibly measure $\delta^{13}\text{C}$ values was 1.5 nmol.

The $\delta^{13}\text{C}$ of reference compounds was determined by using (i) a CE Instruments elemental analyzer (CE Instruments, Rodano, Italy) coupled to a Micromass Isochrom isotope-ratio mass spectrometer (EA-IRMS) and (ii) the GC-C-IRMS instrument described above. In the first case, the compounds were injected as gas phase (ethene and VC) or liquid phase (cDCE, TCE, and PCE) into the syringe injection port of the elemental analyzer. When using the second system (GC-C-IRMS), gas-phase standards were prepared by dispensing the compounds as gas or liquid phase into helium-filled 500 mL glass bottles at final concentrations of 15, 60, and 120 μM per compound. After allowing at least 30 min for evaporation of the compounds added as liquid phase, gas samples were injected into the GC-C-IRMS system at a split ratio of 10:1, which resulted in signal intensities for mass 44 between 0.75 and 6 V. The difference in the average $\delta^{13}\text{C}$ ($n = 3$) between the largest and the smallest peak size was $\leq 0.27\text{‰}$, which indicates a good linearity of the analytical system. Standard uncertainties were $\leq 0.07\text{‰}$ for EA-IRMS measurements ($n = 4$) and $\leq 0.09\text{‰}$ for the GC-C-IRMS ($n = 9$). Values obtained by EA-IRMS and GC-C-IRMS agreed within the range of uncertainty. For PCE, the $\delta^{13}\text{C}$ value was also determined on a conventional dual-inlet isotope-ratio mass spectrometer after combustion of the PCE in breakseals. The value obtained by dual-inlet mass spectrometry ($-27.59 \pm 0.04\text{‰}$) was similar to the values obtained by the continuous-flow systems which were $-27.27 \pm 0.03\text{‰}$ and $-27.20 \pm 0.07\text{‰}$ for EA-IRMS and GC-C-IRMS, respectively. The reference compounds were used to evaluate the occurrence of isotope fractionation during sample treatment and to verify the analytical system on each day $\delta^{13}\text{C}$ analyses were performed.

For carbon isotope analysis of ethene and chlorinated ethenes in field samples and microcosms, headspace techniques were used. To evaluate if aqueous-phase/gas-phase partitioning was accompanied by carbon isotope fractionation, aqueous standards were prepared by adding reference compounds to 125 mL bottles filled with organic free distilled water. After 2 h of stirring, a headspace was introduced by replacing 20 mL of solution with helium. The bottles were placed on a reciprocating shaker at 120 rpm for at least 2 h prior to analysis. For carbon isotope analysis, 0.1–1 mL of headspace gas was injected into the GC-C-IRMS system. The molecules in the headspace were slightly enriched in ^{13}C ($0.33\text{--}0.61\text{‰}$) compared to dissolved molecules (33).

Field samples were similarly treated as the aqueous standards, and 0.1–1.0 mL of headspace gas was injected

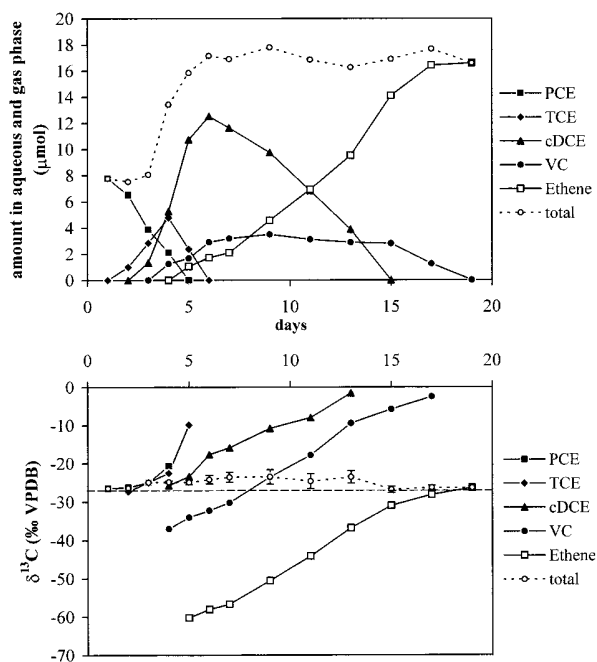


FIGURE 2. Amount of each chlorinated ethene in aqueous and gas phase of microcosm. Carbon isotope ratio ($\delta^{13}\text{C}$) of the initially added PCE (dashed line), of each compound, and of the total of all compounds. The standard uncertainty of the $\delta^{13}\text{C}$ values of individual compounds corresponds approximately to the size of the symbols.

three to four times into the GC-C-IRMS system. In the microcosm study, carbon isotope ratios of chlorinated ethenes were determined by injecting two or three times 0.1–1.0 mL gas phase of the microcosm into the GC-C-IRMS system. Headspace gas concentrations were calculated based on the peak areas of the mass 44 ion trace and external standards. For most of the field and microcosm samples, split injection at a split ratio of 10:1 was used, which resulted in the following lower concentration limits for carbon isotope analysis (1 mL injection): PCE, 12 μM ; TCE, 21 μM ; cDCE, 50 μM ; VC, 8 μM ; and ethene, 1 μM . For concentrations of PCE, TCE, and cDCE below these limits, 0.4 mL of headspace gas was injected in splitless mode, which led to a decrease of the lower concentration limit for these compounds by about a factor of four.

All $^{13}\text{C}/^{12}\text{C}$ ratios are reported in the usual delta notation ($\delta^{13}\text{C}$) referenced to VPDB (Vienna Pee Dee Belemnite). The $\delta^{13}\text{C}$ value is defined as $\delta^{13}\text{C} = (R_s/R_r - 1) \times 1000$, where R_s and R_r are the $^{13}\text{C}/^{12}\text{C}$ ratios of the sample and the standard, respectively. Measured $\delta^{13}\text{C}$ values were corrected for the small carbon isotope fractionation during aqueous-phase/gas-phase partitioning and are reported with the corresponding standard uncertainties (34). Isotope fractionation is expressed as enrichment factors $\epsilon = \delta^{13}\text{C}_o - \delta^{13}\text{C}_p$, where $\delta^{13}\text{C}_p$ is the carbon isotope ratio of the product and $\delta^{13}\text{C}_o$ the carbon isotope ratio of the corresponding precursor. The $\delta^{13}\text{C}$ of the total of all chlorinated ethenes was calculated by multiplying the amount (microcosm) or concentration (field site) of each compound with its $\delta^{13}\text{C}$, adding all contributions and dividing the sum by the total amount or concentration. The standard uncertainty of the $\delta^{13}\text{C}$ of the total was calculated using the law of propagation of uncertainty (34).

Results and Discussion

Microcosm Experiment. PCE Dechlorination. PCE was dechlorinated to cDCE via TCE within 6 days, and cDCE accumulated in the microcosm (Figure 2). VC and ethene were first detected on days 4 and 5, respectively. After 19

TABLE 2. Concentrations and Stable Carbon Isotope Ratios ($\delta^{13}\text{C}$) of Chlorinated Ethenes in Groundwater Samples of the Toronto Site Taken on August 28, 1998^a

monitoring well	PCE concn (μM)	$\delta^{13}\text{C}$ (‰ VPDB)	TCE concn (μM)	$\delta^{13}\text{C}$ (‰ VPDB)	cDCE concn (μM)	$\delta^{13}\text{C}$ (‰ VPDB)	VC concn (μM)	$\delta^{13}\text{C}$ (‰ VPDB)	ethene concn (μM)	$\delta^{13}\text{C}$ (‰ VPDB)	total ^b concn (μM)	$\delta^{13}\text{C}$ (‰ VPDB)
CEL1	4.2	-25.7 \pm 0.4	5.2	-29.5 \pm 0.4	7.2	-31.5 \pm 0.3	0.9	-40.4 \pm 0.3	0.07		17.7	-29.5 \pm 0.3
CEL2	101.8	-31.0 \pm 0.4	19.2	-30.7 \pm 0.4	393.0	-25.8 \pm 0.3	162.4	-40.3 \pm 0.4	37.3	-51.7 \pm 0.3	713.6	-31.3 \pm 0.5
CEL3	325.2	-31.7 \pm 0.5	48.9	-29.9 \pm 0.4	191.5	-26.4 \pm 0.3	45.5	-35.7 \pm 0.2	149.0	-34.2 \pm 0.3	760.0	-30.9 \pm 0.4
CEL10			1.0		1592.4	-15.9 \pm 0.3	1123.5	-33.5 \pm 0.3	736.8	-51.8 \pm 0.4	3453.7	-29.3 \pm 1.0
ISRP 4-1			0.5		156.3	4.2 \pm 0.4	346.9	-18.5 \pm 0.2	693.6	-41.7 \pm 0.3	1197.3	-29.0 \pm 1.0
ISRP 4-2					31.9	5.1 \pm 0.6	90.9	-11.3 \pm 0.3	467.0	-35.4 \pm 0.3	589.8	-29.5 \pm 0.9
ISRP 5-1					5.9		82.7	25.4 \pm 0.2	661.8	-31.9 \pm 0.3	750.5	-25.3 \pm 1.0

^a In addition to the listed compounds small concentrations of 11DCE ($\leq 0.3\%$ of corresponding cDCE concentration), tDCE ($\leq 2.7\%$ of corresponding cDCE concentration), and ethane ($\leq 7\%$ of corresponding ethene concentration) were found in some monitoring wells. ^b The $\delta^{13}\text{C}$ of the total of all chlorinated ethenes and ethene was calculated by multiplying the concentration of each compound with its $\delta^{13}\text{C}$, adding all contributions and dividing the sum by the total concentration.

days, PCE dechlorination to ethene was completed. In addition to the chlorinated ethenes mentioned above, small amounts of tDCE were detected ($<0.5 \mu\text{mol}$). About 55% of the added PCE initially sorbed to the aquifer material and desorbed again during the experiment as indicated by the increase of the total amount of chlorinated ethenes in the aqueous and gas phases. After day 5, the total amount of chlorinated ethenes in the aqueous and gas phases remained constant and corresponded to the initially added amount of PCE (17 μmol).

Carbon Isotope Fractionation during PCE Dechlorination. The $\delta^{13}\text{C}$ values of PCE and the dechlorination products covered a very large range, reaching from -60.2% to -1.5% (Figure 2). The most significant changes in $\delta^{13}\text{C}$ were observed for cDCE (-25.7 to -1.5%), VC (-37.0 to -2.5%), and ethene (-60.2 to -26.4%). In general, the $\delta^{13}\text{C}$ of each dechlorination product was always more negative than the $\delta^{13}\text{C}$ of the corresponding precursor. In addition, the $\delta^{13}\text{C}$ values of each compound increased with time (Figure 2). This suggests that the dechlorination rate was slightly faster for molecules containing ^{12}C than for molecules containing ^{13}C , which led to a depletion of ^{13}C in the product and an enrichment of ^{13}C in the remaining precursor of each dechlorination step. However, the degree of isotope fractionation seems to have been smaller for the first two dechlorination steps, PCE to TCE and TCE to cDCE than for the last two steps from cDCE to VC and VC to ethene. Isotope fractionation during the first dechlorination step might have been slightly larger than it appears in Figure 2 since the $\delta^{13}\text{C}$ of PCE may have been affected by desorption of sorbed PCE with the initial $\delta^{13}\text{C}$ value. The $\delta^{13}\text{C}$ of PCE at day 1 (-26.8%) was close to the $\delta^{13}\text{C}$ of the added PCE (-27.3%) which indicates that no significant isotopic fractionation occurred during sorption of PCE to the aquifer sediments. The $\delta^{13}\text{C}$ values of PCE and that of all intermediate products (TCE, cDCE, and VC) increased above the value of the added PCE. In contrast, the $\delta^{13}\text{C}$ of the final product, ethene, reached the value of the added PCE as dechlorination came to completion. For mass balance considerations, the $\delta^{13}\text{C}$ value of ethene has to correspond to the $\delta^{13}\text{C}$ of the added PCE if dechlorination has reached completion and no final product other than ethene is formed. The calculated $\delta^{13}\text{C}$ of the total of all chlorinated ethenes increased by about 3‰ and reached the initial value again toward the end of the experiment. The increase of the calculated $\delta^{13}\text{C}$ may have been due to the transient production of small amounts of ^{13}C -depleted tDCE.

Estimation of Enrichment Factors. Enrichment factors are usually quantified using the Rayleigh model (7), which predicts $\delta^{13}\text{C}$ values for one-step processes. Except for the first dechlorination step, this model cannot be applied to the data obtained in this study since the $\delta^{13}\text{C}$ values of the intermediate products are affected by a production and a

consumption process which occur simultaneously. However, the degree of isotope fractionation during the different dechlorination steps can roughly be quantified based on the difference in $\delta^{13}\text{C}$ between the initially formed dechlorination product and the corresponding precursor. Initially, the $\delta^{13}\text{C}$ of each intermediate product is mainly controlled by the process producing the intermediate product (TCE, cDCE, and VC). The following enrichment factors were obtained: 2‰ for dechlorination of PCE to TCE (day 2), 4‰ for dechlorination of TCE to cDCE (day 4), 12‰ for dechlorination of cDCE to VC (day 4), and 26‰ for dechlorination of VC to ethene (day 5). In future studies, microcosm experiments will be performed starting with the intermediate products, which will make it possible to constrain the fractionation factors for each dechlorination step using the Rayleigh model. In addition, a multistep fractionation model will be developed.

Field Study. Concentrations and $\delta^{13}\text{C}$ of Chlorinated Ethenes. PCE was detected in groundwater samples of three monitoring wells (CEL1, CEL2, and CEL3) that are located close to the areas where PCE was suspected to have entered the aquifer (Table 2). Two of these monitoring wells (CEL2 and CEL3) are screened at the level of the upper clay unit, one (CEL1) at the level of the upper sand/silt unit (Table 1). In addition to PCE, also TCE, cDCE, VC, and ethene were detected in these three monitoring wells (Table 2). In the other monitoring wells (CEL10, ISRP4-1, ISRP4-2, and ISRP5-1), PCE concentrations were below detection limit and TCE concentrations low. Ethene concentrations at these monitoring wells were higher than in CEL1, CEL2, and CEL3 (Table 2). The $\delta^{13}\text{C}$ values (Table 2) fall within a narrow range for PCE (-31.7% and -25.7%) and TCE (-30.7% to -29.5%) compared to the large variation observed for cDCE (-31.5% to $+5.1\%$), VC (-40.4% to $+25.4\%$), and ethene (-51.7% to -31.9%).

Carbon Isotope Fractionation during PCE Dechlorination. To evaluate if $\delta^{13}\text{C}$ values of chlorinated ethenes change in a systematic way with increasing dechlorination, mole fractions and $\delta^{13}\text{C}$ values of all compounds at each monitoring well were plotted in the order of decreasing mole fraction of PCE and increasing mole fraction of ethene (Figure 3). Differences in $\delta^{13}\text{C}$ among PCE, TCE, and cDCE were small compared to differences among cDCE, VC, and ethene. Ethene was depleted in ^{13}C compared to VC and VC compared to cDCE in samples from all monitoring wells except CEL3. Furthermore, $\delta^{13}\text{C}$ values of cDCE, VC, and ethene increased with increasing degree of dechlorination, similarly to what occurred in the microcosm. While the $\delta^{13}\text{C}$ of cDCE and VC reached values larger than 0‰, the $\delta^{13}\text{C}$ of ethene approached a value that is similar to the $\delta^{13}\text{C}$ of the total of all chlorinated ethenes in each well and possibly corresponded to the $\delta^{13}\text{C}$ of the spilled PCE. The spilled PCE is likely to have a $\delta^{13}\text{C}$

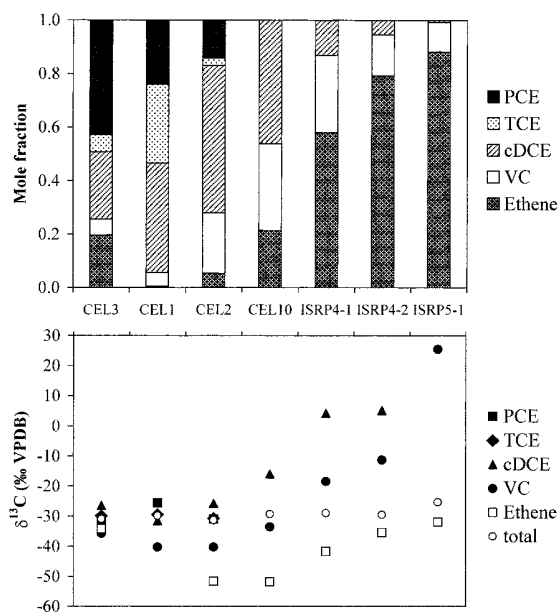


FIGURE 3. Mole fractions and carbon isotope ratios ($\delta^{13}\text{C}$) of chlorinated ethenes in groundwater samples taken at Toronto field site on August 28, 1998. The mole fractions were calculated by dividing the concentration of each compound by the total concentration of chlorinated ethenes. The standard uncertainty of the $\delta^{13}\text{C}$ values is smaller than the size of the symbols (Table 2).

similar to the $\delta^{13}\text{C}$ of PCE at CEL3 (-31.7‰), where the highest fraction of PCE was detected, and similar to the $\delta^{13}\text{C}$ of the sum of all chlorinated ethenes (-29.5 to -31.3‰) in monitoring wells close to the suspected PCE entry points (CEL1, CEL2, and CEL3).

The $\delta^{13}\text{C}$ of PCE at CEL2 and the $\delta^{13}\text{C}$ of ethene at CEL3 deviate from the general trend toward more enriched $\delta^{13}\text{C}$ values with increasing dechlorination. Although CEL2 had the smallest fraction of PCE, the $\delta^{13}\text{C}$ of PCE was depleted in ^{13}C by 5.3‰ compared to PCE at CEL1 and similar to the $\delta^{13}\text{C}$ of PCE at CEL3. At CEL2, isotope fractionation accompanying the first dechlorination step may have been masked by dissolution of PCE during a second contact of the groundwater with PCE in nonaqueous phase after dechlorination of initially dissolved PCE had occurred. Ethene detected at CEL3 was more enriched in ^{13}C than ethene found at other wells with a similar (CEL10) or higher fraction (ISRP4-1) of ethene. The ethene found at CEL3 may have been produced by nearly complete dechlorination of PCE that had been dissolved initially in the groundwater, while the chlorinated ethenes originate from PCE that was dissolved during a more recent contact of the groundwater with PCE in nonaqueous phase. Alternatively, at CEL2 and CEL3 groundwater from different layers, in which dechlorination had progressed to a different degree, might have been mixed during sampling. CEL2 and CEL3 have a longer screened interval (2.5 m) than CEL1 (1.5 m).

Apart from the two exceptions discussed above, the $\delta^{13}\text{C}$ values of all chlorinated ethenes appear to follow a similar trend toward more enriched values with increasing dechlorination, although the monitoring wells are not located along a flowline and total concentrations of chlorinated ethenes vary. This suggests that the degree of isotope fractionation was fairly constant throughout the site and did not depend on the concentration of chlorinated ethenes.

Comparison of Microcosm and Field Study. Both the microcosm and field studies suggest that carbon isotope fractionation during the first two dechlorination steps was small compared to that during the last two dechlorination

steps. Isotope fractionation occurring during the initial dechlorination of PCE may have been partly masked by desorption and subsequent dissolution of PCE with the initial isotope composition. In both studies, a large carbon isotope fractionation during dechlorination of cDCE to VC and VC to ethene could be observed, which led to the occurrence of cDCE and VC that was very enriched in ^{13}C during the last stages of microbial dechlorination. In the field study, cDCE and VC became more enriched in ^{13}C than in the microcosm study. This difference was mainly due to the larger concentrations of chlorinated ethenes at the field site than in the microcosm, which made it possible to determine $\delta^{13}\text{C}$ values in smaller fractions of cDCE or VC in relation to the initial concentration. The smaller the fraction of remaining intermediate product is, the more enriched in ^{13}C it becomes, which explains the higher $\delta^{13}\text{C}$ values for cDCE and VC at some monitoring wells at the field site compared to the microcosm data. The good correspondence of the general carbon isotope pattern observed in the microcosm and field studies suggests that microcosm data can be used for the interpretation of isotope data obtained in field situations. This is an important conclusion for the use of environmental isotopes in organic contaminant studies in groundwater. Further studies will focus on the variability of isotope fractionation between different aquifers and in microcosms derived from different sites.

Implications for Use of $\delta^{13}\text{C}$ To Evaluate Origin and Fate of Chlorinated Ethenes. Two of the major issues confronting consultants and regulators dealing with contaminated groundwater are identification of contaminant sources and evaluation of processes that can attenuate the contaminants within groundwater flow systems. Biodegradation is of particular interest since it is often the only process that may result in the transformation of contaminants to nontoxic products. However, it is often difficult to demonstrate biodegradation based on concentration changes alone, since concentration changes can also be due to physical processes (e.g. dilution and sorption).

Regarding identification of sources, the results of this study suggest that the carbon isotope composition of dissolved PCE close to the source areas seems to preserve the original isotope composition of the spilled PCE. Thus it may be possible to distinguish between different sources of PCE based on the $\delta^{13}\text{C}$ values of dissolved PCE even if dechlorination occurs. However, the most important contribution of this study is the identification of the large carbon isotope fractionation associated with the last two dechlorination steps from cDCE to VC and VC to ethene. The corresponding large enrichment of ^{13}C in cDCE and VC may serve as a powerful tool to demonstrate or verify that complete dechlorination of TCE or PCE occurs at sites undergoing intrinsic bioremediation. The observation that the $\delta^{13}\text{C}$ of the ethene reaches the $\delta^{13}\text{C}$ of the initial substrate may also make it possible to distinguish ethene originating from different contaminants (e.g. PCE and 1,2-dichloroethane) if the contaminants have different initial $\delta^{13}\text{C}$ values and dechlorination has reached completion. In addition, measurement of stable carbon isotope ratios is a promising tool to verify that all byproducts from biodegradation of the original substrate have been included, by calculating the $\delta^{13}\text{C}$ of the sum of the remaining original precursor and all dechlorination products. In conclusion, this study illustrates that compound-specific carbon isotope ratios can serve as a sensitive tool to assess the fate of chlorinated ethenes in aquifers and to verify observations that are based on concentration measurements.

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