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Stable Carbon Isotope Evidence for Intrinsic Bioremediation of Tetrachloroethene and Trichloroethene at Area 6, Dover Air Force Base

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Area 6 at Dover Air Force Base (Dover, DE) has been the location of an in-depth study by the RTDF (Remediation Technologies Development Forum Bioremediation of Chlorinated Solvents Action Team) to evaluate the effectiveness of natural attenuation of chlorinated ethene contamination in groundwater. Compound-specific stable carbon isotope measurements for dissolved PCE and TCE in wells distributed throughout the anaerobic portion of the plume confirm that stable carbon isotope values are isotopically enriched in ^{13}C consistent with the effects of intrinsic biodegradation. During anaerobic microbial reductive dechlorination of chlorinated hydrocarbons, the light (^{12}C) versus heavy isotope (^{13}C) bonds are preferentially degraded, resulting in isotopic enrichment of the residual contaminant in ^{13}C. To our knowledge, this study is the first to provide definitive evidence for reductive dechlorination of chlorinated hydrocarbons at a field site based on the $\delta^{13}C$ values of the primary contaminants spilled at the site, PCE and TCE. For TCE, downgradient wells show $\delta^{13}C$ values as enriched as −18.0‰ as compared to $\delta^{13}C$ values for TCE in the source zone of −25.0 to −26.0‰. The most enriched $\delta^{13}C$ value on the site was observed at well 236, which also contains the highest concentrations of cis-DCE, VC, and ethene, the daughter products of reductive dechlorination. Stable carbon isotope signatures are used to quantify the relative extent of biodegradation between zones of the contaminant plume. On the basis of this approach, it is estimated that TCE in downgradient well 236 is more than 40% biodegraded relative to TCE in the proposed source area.

Introduction

Previously stable carbon isotope analysis focused on the isotopic characterization of CO_2 or dissolved inorganic carbon to assess bioremediation. This approach monitored changes in the $\delta^{13}C$ value of the bulk carbon pool due to the addition of CO_2-derived from biodegradation of hydrocarbon contaminants (*1*, *2*). The results were often ambiguous, however, due to the difficulty in constraining multiple subsurface sources and sinks of CO_2 and due to the effects of indigenous respiration, which can mask changes in $\delta^{13}C$ values of the bulk carbon pool related to contaminant degradation unless such degradation rates are very high.

High sensitivity GC/C/IRMS (gas chromatograph/combustion/isotope ratio mass spectrometry), through its ability to characterize the stable carbon isotope composition of individual compounds at low dissolved concentrations in groundwater, provides a new tool for investigation of hydrocarbon contamination. Early studies focused on characterizing differences in the isotopic signatures of contaminants from different sources in order to establish the potential range in $\delta^{13}C$ values (*3*−*7*). Provided that this isotopic signature is conserved in the subsurface, stable carbon isotope analysis has the potential to identify different sources of contamination. Laboratory experiments have assessed the kinetic carbon isotope effects associated with key subsurface processes controlling contaminant fate and transport in groundwater. These studies showed that stable carbon isotope values are indeed largely conserved during nondegradative processes such as sorption (*7*, *8*), dissolution (*4*, *9*) and volatilization (*7*, *9*, *10*). Carbon isotope fractionation associated with these processes is small and, in many cases, less than analytical uncertainty.

In contrast, laboratory studies have shown that for chlorinated hydrocarbons, degradation can involve large and reproducible kinetic isotope effects, producing systematic changes in the $\delta^{13}C$ values of the residual contaminant (*11*−*16*). During anaerobic microbial reductive dechlorination of chlorinated hydrocarbons, the light (^{12}C) versus heavy isotope (^{13}C) bonds are preferentially degraded, resulting in isotopic enrichment of the residual contaminant in ^{13}C. Laboratory studies have shown that the magnitude of fractionation decreases with increasing degree of chlorination of the chlorinated ethene. Maximum isotopic enrichment in the residual contaminant relative to its initial isotopic composition of up to 5−6‰ has been observed for experiments involving degradation of tetrachloroethene (PCE) (*15*, *17*). Enrichments of up to 14‰ have been observed for experiments focusing on degradation of trichloroethene (TCE) (*13*, *15*−*17*). Experiments that characterized the isotopic composition of both the degrading parent compound and daughter products indicate that, when initially produced, daughter products are more isotopically depleted in ^{13}C than the parent compound, reflecting the preferential biodegradation of light ^{12}C-containing molecules. The daughter products begin to show the characteristic isotopic enrichment trends very quickly, however, as they themselves undergo subsequent biodegradation to the next less chlorinated compound (*13*, *15*, *17*).

In contrast to the source differentiation application described above, the objective of this study was to determine if isotopic fractionation and the enrichment trends produced by biodegradation in the laboratory during reductive dechlorination of PCE and TCE could be used as a means of

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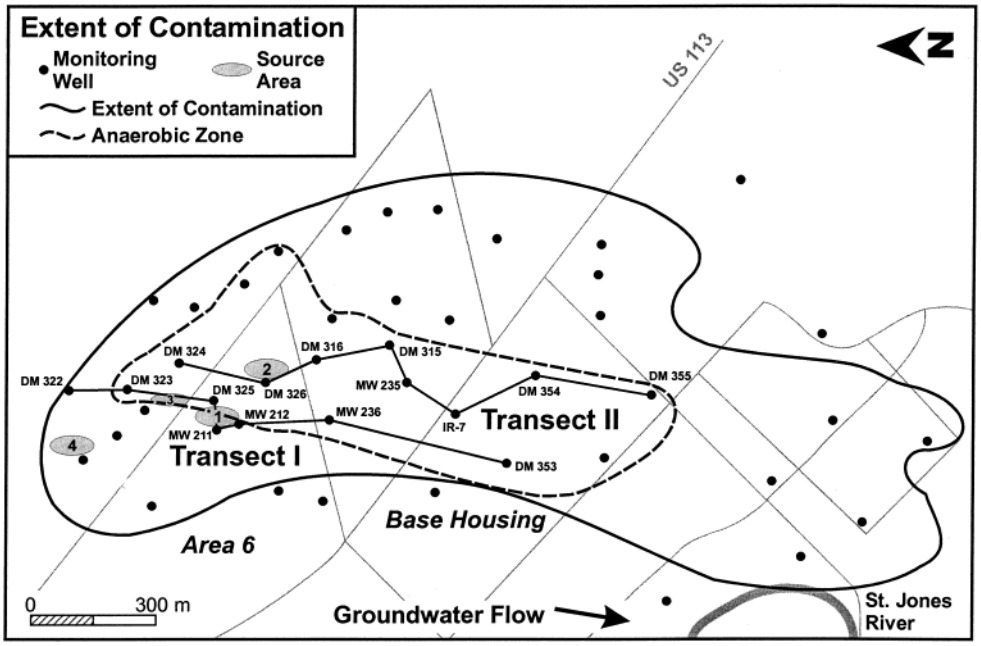


FIGURE 1. Site map of Area 6, Dover Air Force Base, showing names of wells in the anaerobic part of the plume sampled for the isotopic study. Source areas identified in previous work are shown as ovals (*20*). The lateral extent of contamination of the deep aquifer is also shown. Transect I refers to a sampling transect approximately downgradient and upgradient of source area 1 (wells 211/212), encompassing downgradient wells 236 and 353, and upgradient wells 325, 323, and 322. Transect II refers to a sampling transect approximately downgradient and upgradient from source area 2 (well 326), encompassing downgradient wells 316, 315, 235, ir7, 354, 355, and upgradient well 324.

identifying and monitoring intrinsic biodegradation in the field. Sturchio et al. (*18*) used variations in stable Cl isotope ratios of TCE to evaluate natural attenuation in an aerobic aquifer. Hunkeler et al. (*15*) focused on using the large carbon isotope fractionation associated with cis-DCE, VC, and ethene to verify that extensive reductive dechlorination was occurring in a PCE-contaminated aquifer. To our knowledge, the present study is the first to provide definitive evidence for reductive dechlorination of chlorinated hydrocarbons at a field site based on the $\delta^{13}C$ values of the primary contaminants spilled at the site, PCE and TCE.

Site Background

Area 6 at Dover Air Force Base (Dover, DE) is part of the industrial portion of the base where solvents were commonly used for aircraft and vehicle maintenance, painting, stripping, and welding. Spills and past waste disposal practices from multiple sources of varying and unknown ages resulted in introduction of a variety of contaminants into the groundwater. While some petroleum hydrocarbon sources exist, the majority of the contamination at the identified source areas consisted of chlorinated solvents, both PCE and TCE (*19*, *20*). While Area 6 contains a number of different source areas and resultant plumes, this study focused on two major source areas (1 and 2) and the contamination upgradient and downgradient from these source areas (Figure 1). Since 1995, Area 6 has been the location of an in-depth study by the RTDF to evaluate the effectiveness of intrinsic bioremediation (natural attenuation) of PCE and TCE contamination in groundwater and sediments. This location was chosen because of its relatively homogeneous geology (12−17 m of unconsolidated Pleistocene coarse sands and gravels—the Columbia Formation), the presence of a shallow aquifer within these deposits underlain by a confining layer, and indications that sufficient biological activity exist in the aquifer (*19*, *20*). Groundwater flows from a recharge area in the northern portion of the study area toward the Saint Jones River to the south and southwest at a rate of approximately 14.4 m/year (Figure 1) (*21*).

Summary of Conceptual Model for the Site. Results of the geological, hydrogeological, and biogeochemical study and computer modeling of the site have been previously published (*20*, *21*). Introduction of PCE and TCE into the permeable soil overlying the aquifer resulted in vertical migration of DNAPL (dense nonaqueous phase liquid) to the deep zone of the aquifer (*21*, *22*). As PCE and TCE were the only chlorinated solvents used in Area 6, the presence of daughter products (cis-DCE, VC, and ethene) in the deep aquifer is indicative of biological reductive dechlorination. Additional lines of biogeochemical evidence for intrinsic bioremediation via reductive dechlorination include the delineation of the three plumes of daughter products entirely within the PCE and TCE plumes and largely within the anaerobic zone of the aquifer. Within the anaerobic zone, increasing soluble chloride ion concentrations correlate with decreases in chlorinated ethene concentrations, and high dissolved H_2 levels (>10nM) correlate with high dissolved CH_4 concentrations (100−500 μg/L) and depleted levels of dissolved oxygen (<1 mg/L). These trends support a conceptual model of biological reductive dechlorination of chlorinated ethenes within the anaerobic zone of the plume (*20*). Downgradient of the anaerobic zone (Figure 1), the groundwater is reoxygenated due to infiltration of surface water and mixing. Aerobic biodegradation of the ethene, VC, and to a lesser extent cis-DCE occurs within this zone. Most of the PCE has already been removed by reductive dechlorination within the anaerobic zone, but any residual TCE persists throughout the aerobic part of the plume, since unlike the daughter products it does not readily degrade under aerobic conditions (*20*, *21*).

Methodology

In 1998, we tested the ability of stable carbon isotope analysis to validate the model of anaerobic reductive dechlorination outlined by Klier et al. (*20*) and Clement et al. (*21*). Samples were collected in November 1998 from 15 deep zone wells in the vicinity of source areas 1 and 2 and approximately upgradient and downgradient from these sources areas

(Figure 1). Figure 1 provides a schematic of the overall extent of contamination of the deep aquifer throughout Area 6 and the base housing south of it. The rest of the figures in this study focus only on the source areas and plumes that were the subject of the isotope study specifically and, hence, are simplified schematics of the western plume. Transect I refers to a sampling transect approximately downgradient and upgradient of source area 1 (wells 211/212), encompassing downgradient wells 236 and 353, and upgradient wells 325, 323, and 322 (Figure 1). Transect II refers to a sampling transect approximately downgradient and upgradient from source area 2 (well 326), encompassing downgradient wells 316, 315, 235, ir7, 354, 355, and upgradient well 324. The study focused on the anaerobic zone of the plume, although wells DM 354 and 355 may be close to the transition zone between anaerobic and aerobic conditions. Two additional source areas are located upgradient of source area 1 and are indicated in Figure 1 as source areas 3 (directly upgradient) and 4 (to the northwest of source area 1). Samples were collected from each monitoring well using dedicated bladder pumps and Teflon-lined polyethylene tubing. Wells were pumped at a rate (<1 L/min) until turbidity was visibly equilibrated. The polyethylene tubing was then connected to an in-line flow cell, and field parameters (dissolved oxygen, pH, temperature, specific conductance, and redox potential) were recorded until they stabilized, after which separate samples were collected for isotopic and geochemical analysis.

Groundwater samples for geochemical analysis of organic constituents were collected in VOA vials (headspace free), packed on ice, and shipped via overnight delivery to Lancaster Laboratories, Lancaster, PA. Volatile organic carbon (VOC) analyses were conducted in accordance with U.S. EPA SW-846 Method 8260A using gas chromatography and mass spectrometry. Dissolved oxygen levels ranged from 0.4 to 2.8 mg/L, and ORP ranged from 93 to 322 (mV) with the highest values for both parameters found at well 355 in the anaerobic/aerobic transition zone. Temperature and pH were relatively uniform throughout the site with values between 14.2 and 17.4 °C and between pH 4.9 and pH 5.9, respectively. Conductivity varies from 99 to 225 μmho. No samples for geochemical analysis were available for well 322.

Samples for isotopic analysis were collected as per standard VOC samples in glass VOA sample vessels (headspace free) fitted with Teflon-coated septa. Samples were kept cold (4 °C), shipped to University of Toronto, and analyzed within 1 week. Sample extraction from solution was by headspace equilibration following the protocol described in Slater et al. (*9*). Samples were transferred from sample vials to the analytical system by a gas-tight syringe. Compound-specific carbon isotope analysis of all chlorinated ethenes was carried out by gas chromatograph/combustion/isotope ratio mass spectrometer (GC/C/IRMS). The analytical system consists of a Varian 3400 gas chromatograph equipped with a 30 m × 0.25 mm i.d. DB-624 column for separation of individual compounds. The temperature was held at 40 °C for 4 min, followed by a ramp to 90 °C at 10 °C/min, with a final holding time at 90 °C of 3 min. In real time, individual compounds and He carrier gas eluting from the GC were carried via silica capillary tubing into a 980 °C combustion furnace for combustion to CO_2 and H_2O on a Cu/Ni/Pt catalyst. Water was removed via a semipermeable membrane. The remaining gas stream (CO_2 derived from combustion and He) was introduced into a Finnigan MAT 252 gas source isotope ratio mass spectrometer via silica capillary tubing. The mass spectrometer provides compound-specific stable carbon isotope analysis, i.e., real time measurement of the $^{13}C/^{12}C$ ratio of the CO_2 derived from combustion of each of the individual chlorinated ethenes. $^{13}C/^{12}C$ ratios are expressed as $\delta^{13}C$ signatures where

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000 \quad \text{(in units of ‰)} \tag{1}$$

R_{sample} is the $^{13}C/^{12}C$ ratio in a given compound that is normalized to $R_{standard}$, the $^{13}C/^{12}C$ ratio in a standard reference material. In this study, all $\delta^{13}C$ values are reported relative to the V-PDB standard (*23*). The fractionation factor (α) between any two compounds a and b is defined as

$$\alpha = (1000 + \delta^{13}C_a)/(1000 + \delta^{13}C_b) \tag{2}$$

Samples were run against external CO_2 isotopic standards. Internal reproducibility based on triplicate injections of samples and standards is generally <0.3‰. However, a range of split settings on the split/splitless injector of the GC was used to control the moles of carbon entering the system and to avoid oversaturating the source. Different split settings introduce additional variability. Hence, errors of 0.5‰ for carbon isotope values were used in this study, which incorporate both reproducibility and accuracy of the measurement.

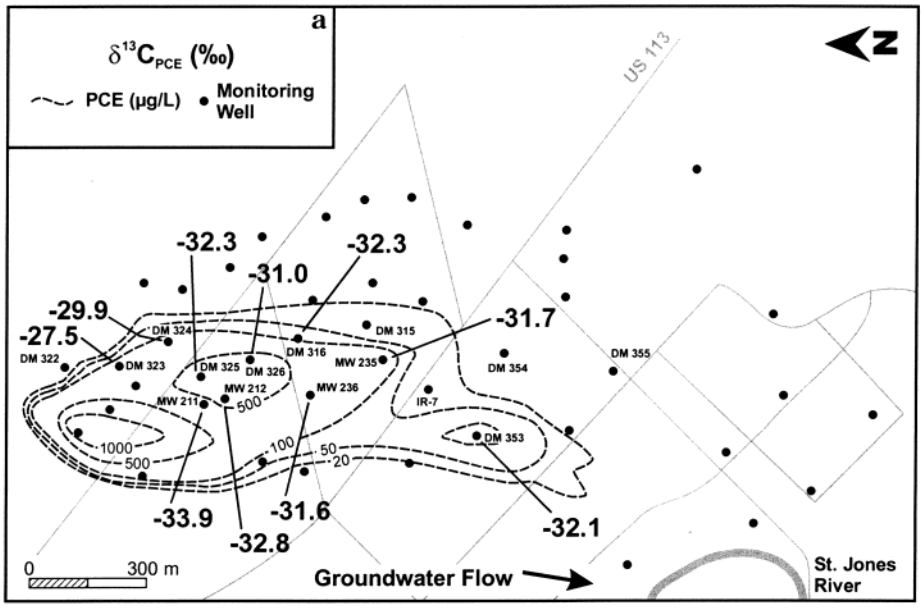
TABLE 1. Concentrations (μg/L) and $\delta^{13}C$ Values (‰) for Chlorinated Ethenes from Wells in Area 6, Dover Air Force Base[a]

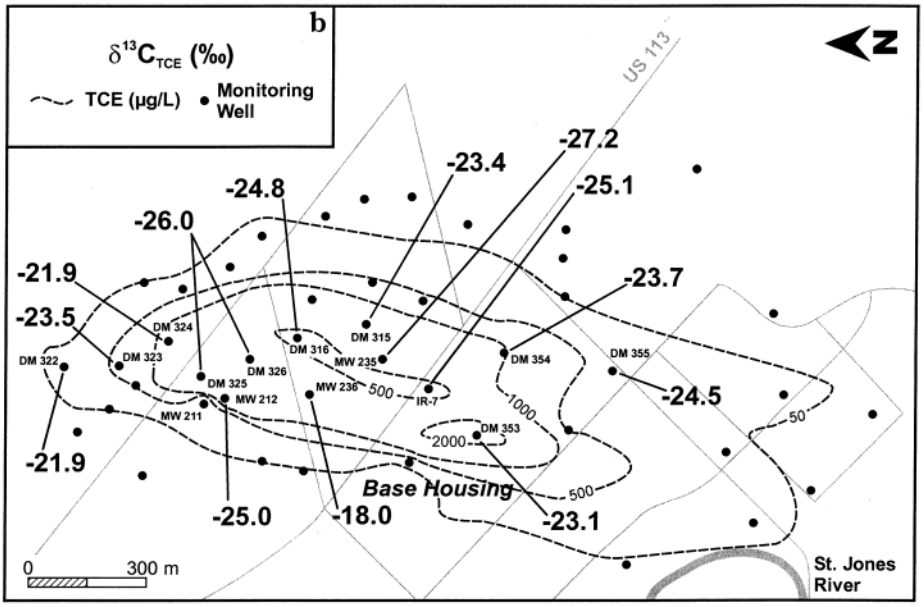
	concns (μg/L)					$\delta^{13}C$ value (‰)	
well	PCE	TCE	cis-DCE	VC	ethene	PCE	TCE
Transect I							
DM 322	na	na	na	na	na	nd	−21.9
DM 323	215	792	169	3.4	<dl	−27.5	−23.5
DM 325	1010	1070	202	<dl	<dl	−32.3	−26.0
MW 211	**249**	**58.6**	**6.5**	**<dl**	**<dl**	**−33.9**	**nd**
MW 212	**541**	**1590**	**922**	**70.0**	**<dl**	**−32.8**	**−25.0**
MW 236	221	1230	23 100	6030	33.0	−31.6	−18.0
DM 353	188	3010	4 830	367	<dl	−32.1	−23.1
Transect II							
DM 324	81.1	1080	3 990	700	11.0	−29.9	−21.9
DM 326	**779**	**1270**	**8 020**	**64.0**	**<dl**	**−31.0**	**−26.0**
DM 316	92.5	593	217	5.3	1.2	−32.3	−24.8
DM 315	<5	1620	279	95	<dl	nd	−23.4
MW 235	357	1540	189	<dl	1.1	−31.7	−27.2
IR7	15.6	504	1 090	596	1.4	nd	−25.1
DM 354	2.9	1050	199	11.8	<dl	nd	−23.7
DM 355	0.8	245	32.2	<dl	<dl	nd	−24.5

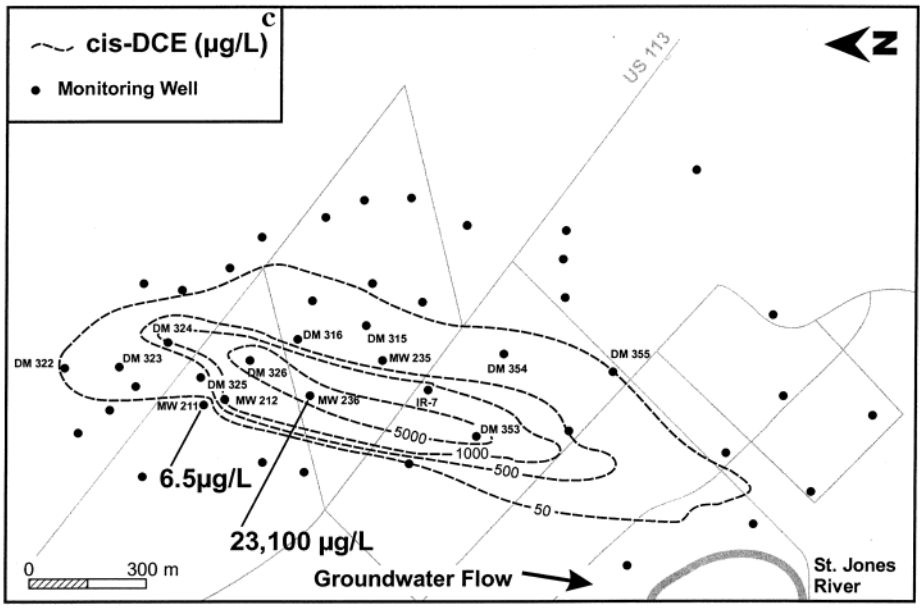
[a] All samples taken in November 1998. Wells closest to source area 1 (211/212) and source area 2 (326) are indicated in bold. na, not analyzed. nd, below detection limit for isotopic analysis. <dl, less than the detection limit.

Results and Discussion

While DNAPL residual in pores and PCE/TCE desorption provides a continuous source of contaminants to the groundwater, there is currently no evidence for the presence of continuous free-phase DNAPL pools at the site. Hence, the original $\delta^{13}C$ signature of the solvent spilled at the various source areas could not be estimated. This information is not essential, however, since the objective of the study is to investigate the extent of biodegradation of upgradient and downgradient wells relative to the source area wells. The well nearest source area 2 (326) and the two wells in source area 1 (211/212) directly adjacent to industrial waste basins do indeed have among the highest concentrations of dissolved PCE and TCE and the least enriched (most negative) $\delta^{13}C$ signatures at the site (Table 1). This evidence supports the assumption that these source wells are the least affected by the isotopic fractionating processes of biodegradation. PCE $\delta^{13}C$ values range from −33.9‰ to −32.8‰ for source


a
δ13CPCE (‰)
PCE (µg/L)
Monitoring Well
N
US 113
-32.3
-32.3
-31.0
-29.9
-27.5
-31.7
DM 322
DM 323
DM 324
DM 325
DM 326
DM 316
DM 315
MW 235
DM 354
DM 355
MW 212
MW 236
MW 211
IR-7
DM 353
500
1000
100
50
20
-33.9
-32.8
-31.6
-32.1
0
300 m
Groundwater Flow
St. Jones River


b
δ13CTCE (‰)
TCE (µg/L)
Monitoring Well
N
US 113
-27.2
-23.4
-25.1
-24.8
-26.0
-23.7
-21.9
-23.5
DM 322
DM 323
DM 324
DM 325
DM 326
DM 316
DM 315
MW 235
DM 354
DM 355
MW 212
MW 236
MW 211
IR-7
DM 353
-24.5
50
500
1000
2000
-21.9
-25.0
-18.0
-23.1
Base Housing
0
300 m
St. Jones River


c
cis-DCE (µg/L)
Monitoring Well
N
US 113
DM 322
DM 323
DM 324
DM 325
DM 326
DM 316
DM 315
MW 235
DM 354
DM 355
MW 212
MW 236
MW 211
IR-7
DM 353
5000
1000
500
50
6.5µg/L
23,100 µg/L
0
300 m
Groundwater Flow
St. Jones River

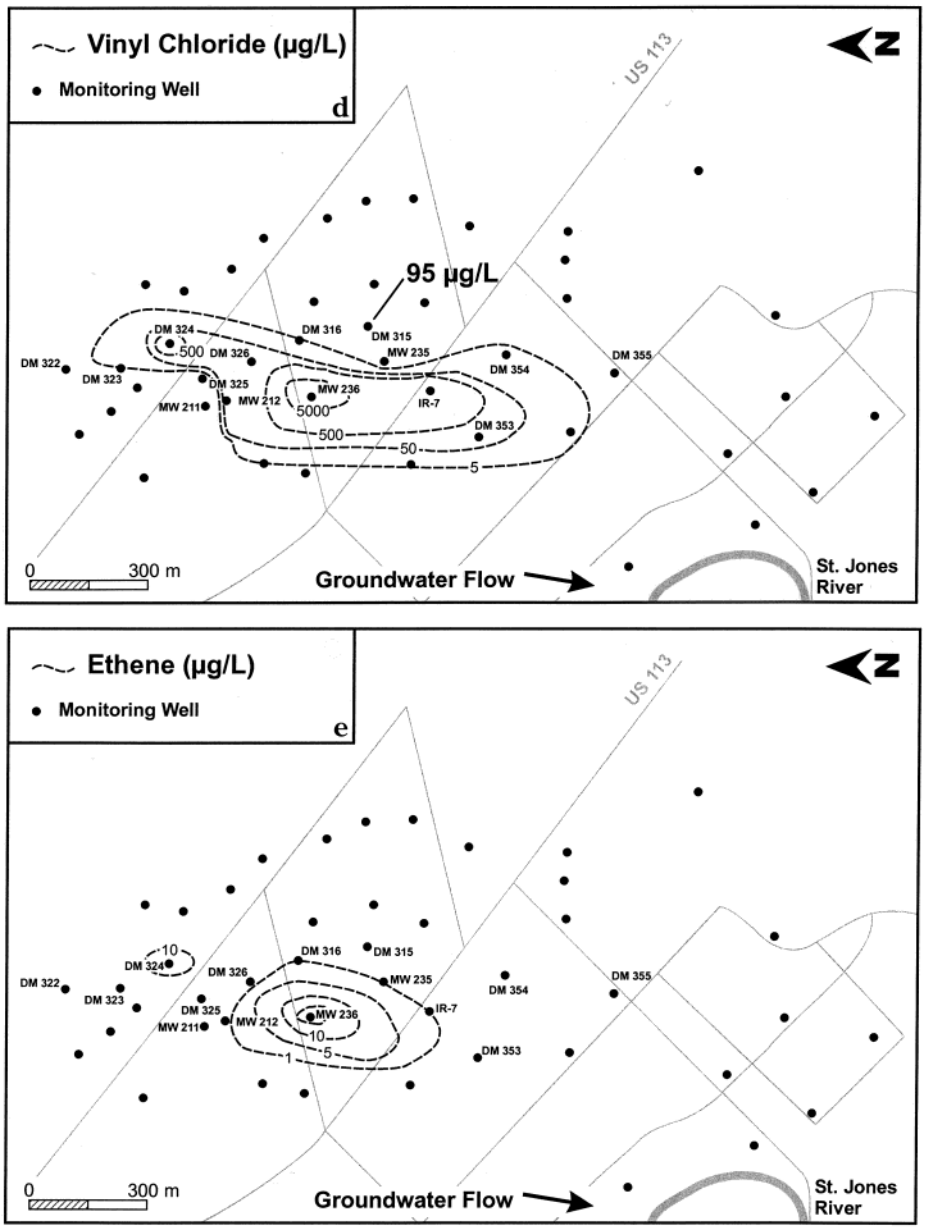


FIGURE 2. (a) Concentrations (μg/L) of dissolved PCE and δ^{13}C values (in ‰) for PCE in groundwater samples taken from wells in the anaerobic part of the plume sampled for the isotopic study. (b) Concentrations (μg/L) of dissolved TCE and δ^{13}C values (in ‰) for TCE sampled as in panel a. (c) Concentrations (μg/L) of dissolved cis-DCE sampled as in panel a. (d) Concentrations (μg/L) of dissolved VC sampled as in panel a. (e) Concentrations (μg/L) of dissolved ethene sampled as in panel a. For all of the above, concentration contours are based on samples taken on the same sampling date as the isotope samples (November 1998) and provide a simplified schematic of the western plume.

area 1 (mean value, −33.4‰) versus −31.0‰ for source area 2 (well 326) (Table 1; Figure 2a). δ^{13}C values for TCE are −26.0‰ (well 326) for source area 2 and −25.0‰ (well 212) for source area 1 (Table 1; Figure 2b). TCE concentrations were below detection limit for isotopic analysis at well 211. The presence of significantly higher concentrations of daughter products at well 212 versus 211 (Table 1; Figure 2c−e) suggests that the estimate of $\delta^{13}C_{TCE}$ for source area 1 based on well 212 alone may be too enriched due to relatively more biodegradation already occurring at well 212 versus well 211. Any error involved in this assumption will only result in a more conservative estimate of the degree of biodegradation in downgradient wells however.

Figure 3a−c shows the mole fractions of the chlorinated ethenes and the δ^{13}C values for PCE and TCE for Transect I. Downgradient wells 236 and 353 exhibit a marked increase in the mole fraction of daughter products (Figure 3a), a slight enrichment in δ^{13}C values for PCE with respect to source wells (211/212) (Figure 3b), and a significant enrichment in δ^{13}C values for TCE with respect to source well 212 (Figure 3c). Consistent with laboratory results (*15*, *17*), these field samples show a larger isotopic enrichment associated with dechlorination of TCE than for PCE. All parameters indicate significant biodegradation of PCE and TCE in wells 236 and 353 with respect to the source area wells. It is particularly notable that the well (236) with the most dramatically enriched δ^{13}C value (−18.0‰) is indeed the most biologically active well at the site based on it having the highest concentrations of all the biological daughter products (Table 1; Figure 2c−e). For upgradient well 325, the isotopic values for both PCE and TCE are within error of those of the source area wells. This well is located very close to source area 1 (Figure 1) and directly adjacent to the industrial waste basins. δ^{13}C values for PCE and TCE in the other upgradient wells

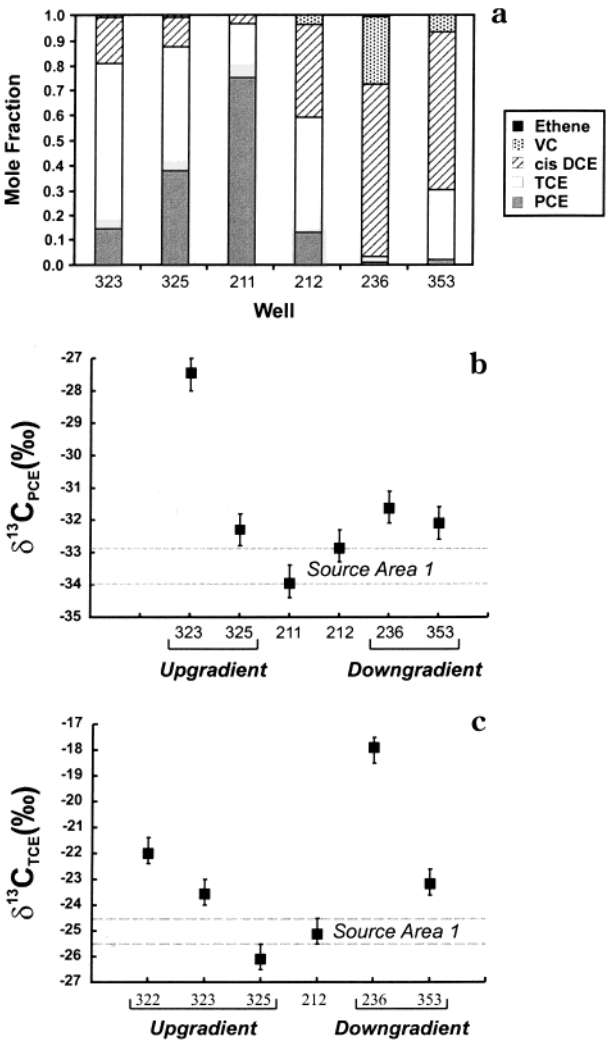


FIGURE 3. Mole fractions (a) and stable carbon isotope values (δ^{13}C in ‰) for dissolved (b) PCE and (c) TCE for Transect I. δ^{13}C values for source area 1 are based on the mean of wells 211/212 (−33.4‰) and 212 (−25.0‰) for PCE and TCE, respectively.

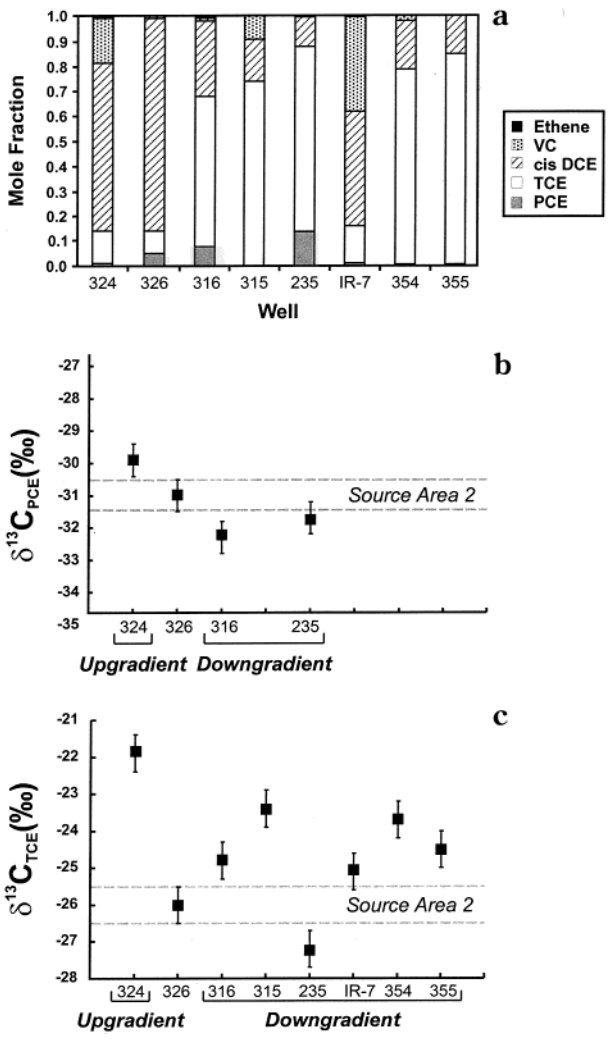


FIGURE 4. Mole fractions (a) and stable carbon isotope values (δ^{13}C in ‰) for dissolved (b) PCE and (c) TCE for Transect II. δ^{13}C values for source area 2 are based on well 326 with values of −31.0‰ and −26.0‰ for PCE and TCE, respectively

(323, 322) are, like the downgradient wells, all resolvably enriched in ^{13}C relative to the source area wells. This isotopic enrichment in both compounds is again consistent with the effects of biodegradation. This interpretation is supported by the increase in the mole fraction of daughter products in well 323 (Figure 3a). Unfortunately, no daughter product data are available for well 322.

Figure 4a−c shows the mole fractions of the chlorinated ethenes and the δ^{13}C values for PCE and TCE for Transect II. For $\delta^{13}C_{PCE}$ values, current detection limits for the isotopic analytical technique meant that δ^{13}C values were only obtained for two downgradient wells and one upgradient well in addition to the source area well (326) (Figure 4b). All three $\delta^{13}C_{PCE}$ values are close to the value of −31.0‰ determined for the source area well within error. Hence $\delta^{13}C_{PCE}$ values do not show a conclusive isotopic enrichment as compared to the source area for this transect.

In contrast, the values obtained for $\delta^{13}C_{TCE}$ in Transect II show definitive evidence of biodegradation effects (Figure 4c) consistent with the larger isotopic fractionation associated with TCE dechlorination versus PCE dechlorination. The single upgradient well (324) exhibits both an increase in the mole fraction of daughter products (Figure 4a) and a more than 4‰ enrichment in the δ^{13}C value for TCE with respect to the source area well (326). With the exception of well 235, all downgradient wells also show a slight to significant enrichment in $\delta^{13}C_{TCE}$ with respect to the source, reflecting the preferential biodegradation of ^{12}C-containing molecules and enrichment of the residual in ^{13}C. The exception is well 235. The depleted δ^{13}C value for TCE for well 235, and the increase in the absolute concentration and the mole fraction of PCE in this well relative to other wells in this area of the plume (Figure 4a) suggest an additional source of solvent in this area. While most other downgradient wells do show an increase in the mole fraction of daughter products relative to the source well (Figure 4a), a simple relationship between isotopic enrichment and mole fraction is complicated by the location of a number of these downgradient wells close to the anaerobic−aerobic transition (wells 354 and 355). Re-oxygenation of the groundwater due to surface water infiltration in this portion of the plume results in aerobic biodegradation of the ethene, VC, and, to a lesser extent, cis-DCE. Since TCE is not significantly degraded under aerobic conditions, there is a disproportionate increase in the mole faction of TCE in these two wells (*20*).

Notably, for both PCE and TCE, the upgradient wells (324, 323, 322) have the most isotopically enriched δ^{13}C values at the site (Figure 2a,b). This significant difference in isotopic signatures with respect to the source wells may be in part due to contributions from additional source areas to the plume in this upgradient area, such as source area 3 or 4. Nonetheless, the isotopic results indicate that the PCE and

TCE in this area of the site is significantly biodegraded. This is supported by both the isotopic enrichment of the PCE and TCE in these wells and the high concentrations of daughter products found in wells 323 and 324 (Table 1; Figures 3a and 4a) and indicates that intrinsic biodegradation is also occurring upgradient of the primary source areas examined in this study. Samples for concentration analysis were not available for well 322 at this sampling date.

Relationship of Isotopic Enrichment Trends to Concentrations of Chlorinated Ethenes. With the exception of well 235, all downgradient wells in both Transects I and II are enriched in $\delta^{13}C_{TCE}$ with respect to their source area wells. In addition, both $\delta^{13}C_{PCE}$ and $\delta^{13}C_{TCE}$ values in all upgradient wells are also enriched in ^{13}C. These results support the interpretation that intrinsic bioremediation is attenuating dissolved PCE and TCE concentrations at the site. That said, the presence of multiple sources of contaminant and competing processes such as dilution, adsorption, and dispersion at this complex site make accurate mass balances difficult. Not surprisingly, there is no precise correlation between decreasing concentrations of PCE and TCE and isotopic enrichment in ^{13}C in these compounds. The best correlations exist when the most direct indicators of the effects of biodegradation are compared, e.g., $\delta^{13}C_{TCE}$ values, and the appearance of key daughter products. Figure 5a–c show a positive correlation between $\delta^{13}C_{TCE}$ values and concentrations of cis-DCE, VC, and ethene with correlation factors (r^2) of 0.56, 0.76, and 0.90, respectively. The fact that these correlations persist despite the complexity of the Dover site underscores their strength and indicates the potential for using $\delta^{13}C$ values as a novel, complementary approach for assessing monitored natural attenuation.

Implications for Validation of Intrinsic Bioremediation

In microcosm experiments using sediments and groundwater from a PCE-contaminated aquifer, Hunkeler et al. (*15*) reported isotopic enrichments in the residual PCE of several ‰. Dramatic shifts in the $\delta^{13}C$ values of all the biological breakdown products were also reported as each daughter product first appeared and then was itself degraded. From the beginning to the end of their experiments, overall changes in the isotopic signatures of cis-DCE, VC, and ethene of >20‰, >35‰, and >30‰ were reported. Unfortunately, since the isotopic signatures of these daughter products are affected by both production and consumption simultaneously during the experiment, it was not possible to completely constrain the degree of isotopic fractionation involved in each step of the dechlorination reaction using these data.

In experiments using a methanogenic microbial consortium (KB-1) enriched from a contaminated field site in southern Ontario, Slater et al. (*17*) were able to obtain the data necessary to constrain fractionation factors for each dechlorination step by doing a series of degradation experiments starting with PCE, TCE, cis-DCE, and VC, respectively. Since each fractionation factor was then determined for a single step in the sequential dechlorination process, the relationship between the concentration of the residual contaminant (or fraction remaining, *f*) and the isotopic composition of the residual contaminant ($\delta^{13}C_{contaminant}$) can be described by a simple Rayleigh closed system set of equations expressed in δ ‰ notation after Mariotti et al. (*24*):

$$(\alpha - 1) \ln f = \ln [(\delta^{13}C_{contaminant}/1000 + 1)/(\delta^{13}C_o/1000 + 1)] \quad (3)$$

where α is the isotopic fractionation factor, $\delta^{13}C_o$ is the initial isotopic composition of the contaminant, and $\delta^{13}C_{contaminant}$ is the isotopic composition of the contaminant after biodegradation. Fractionation factors of 0.9945, 0.9862, 0.9796, and 0.9776 were determined for reductive dechlorination of PCE to TCE, TCE to cis-DCE, cis-DCE to VC, and VC to ethene, respectively. Four replicate experiments yielded the same fractionation factors within error. These values for α are calculated by plotting the data for the biodegradation experiments on a plot of ln *f* versus ln $[(\delta^{13}C_{contaminant}/1000 + 1)/(\delta^{13}C_o/1000 + 1)]$ after Mariotti et al. (*24*) and determining the slope ($\alpha - 1$) by least squares regression of the data.

To date, only two other laboratory studies have reported fractionation factors for chlorinated ethenes during microbial reductive dechlorination. Sherwood Lollar et al. (*16*) reported

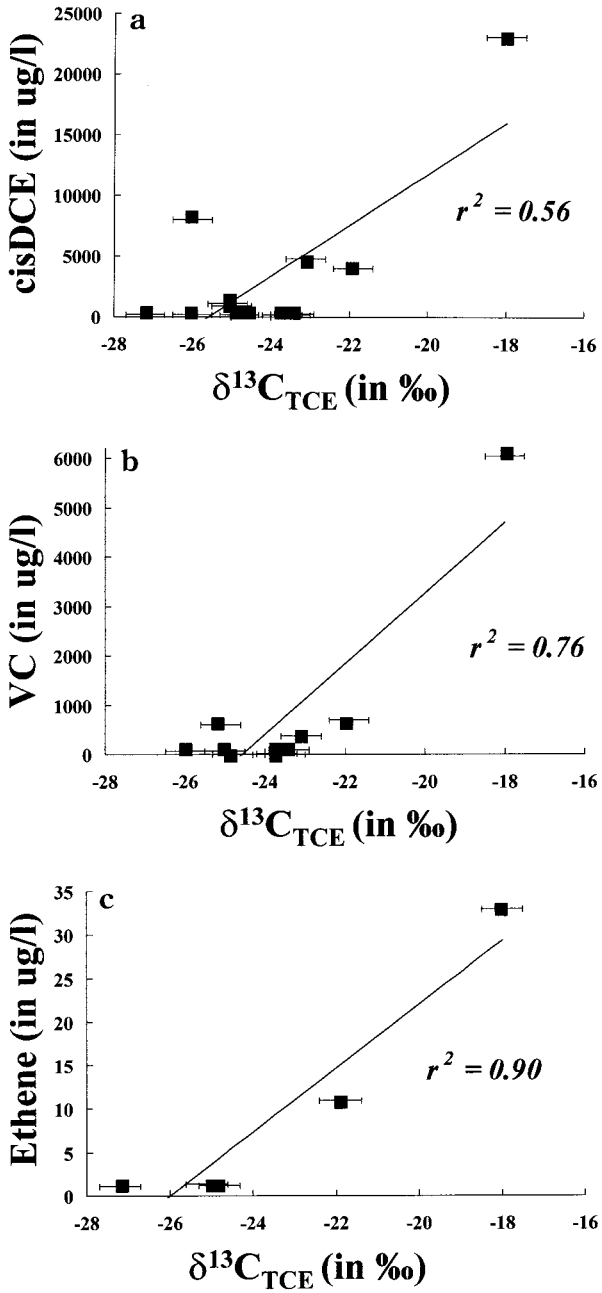


FIGURE 5. $\delta^{13}C$ values (in ‰) for dissolved TCE versus (a) concentrations (μg/L) of dissolved cis-DCE, (b) concentrations (μg/L) of dissolved VC, and (c) concentrations (μg/L) of dissolved ethene. The positive correlations between the degree of isotopic enrichment of TCE and concentrations of key daughter products provide additional evidence for reductive dechlorination at Area 6, Dover Air Force Base.

an α value of 0.9929 for TCE degradation by a mixed facultatively anaerobic consortium enriched from contaminated soil from a site in the southern United States. Bloom et al. (*13*), using subcultures from the same KB-1 microbial consortium as Slater et al. (*17*), obtained a value of α (0.9934) for TCE biodegradation in their first experiment, very similar to the Sherwood Lollar et al. (*16*) estimate. A subsequent duplicate experiment yielded a significantly different value of $\alpha = 0.9975$. The authors note however that this may be due to inadvertent nutrient limitation in the microcosm during this duplicate experiment (*13*). Hence, while results to date indicate the potential for significant variability in α, most reported values of α for TCE microbial reductive dechlorination fall within a range from 0.9862 to 0.9934.

For the Dover AFB site, the best estimate of the amount of biodegradation (*f*) using eq 3 would likely be based on fractionation factors (α) determined for the specific microbial population at the site through experimentation. However, in the absence of such information, an estimate of the extent of biodegradation can be determined using the range of α values available to date. Using eq 3 and the values of source well 212 (−25.0‰) and of downgradient well 236 (−18.0‰) for $\delta^{13}C_o$ and $\delta^{13}C_{contaminant}$, we obtain estimates of 40.5%, 63.5%, and 66.2% biodegradation of the TCE at well 236 relative to the TCE in the source well for $\alpha = 0.9862$ (*17*), 0.9929 (*16*), and 0.9934 (*13*), respectively. This approach assumes that the TCE at well 236 is derived from that in source area 1 (212) and therefore that the isotopic difference in TCE between the two wells is due solely to biodegradation and not to mixing with a different source of TCE. It is a conservative estimate, however, since any additional source of TCE would likely be isotopically lighter than the biodegraded TCE residual and hence would lead to an underestimation of the amount of biodegradation of TCE between the two wells.

Despite the range in reported fractionation factors for TCE microbial reductive dechlorination by different consortia and subcultures (*13*, *16*, *17*), the estimates of 40.5−66.2% biodegradation based on the values reported to date are in relatively good agreement. Better quantification of the extent of biodegradation between wells at a given site would require (i) characterization of the appropriate fractionation factor for the in situ microbial population of dechlorinators and (ii) verification of the isotopic estimate with groundwater flow and transport model estimates of the extent of biodegradation. Nonetheless, the approach is already able to provide an estimate of the relative extent of biodegradation between zones of the contaminant plume. Despite the uncertainty in the fractionation factor, estimates of α based on three different studies indicate that TCE at well 236 is greater than 40% more degraded than TCE in the proposed source area (well 211/212).

All results so far indicate that the extent of carbon isotope fractionation associated with microbial reductive dechlorination decreases with increasing extent of chlorination of the compound. Due to this, Hunkeler et al. (*15*) suggested using the strong enrichment in ^{13}C observed in the daughter products cis-DCE and VC to monitor intrinsic bioremediation due to microbial dechlorination. Their study at a PCE-contaminated field site in southern Ontario showed a large variation in isotopic signatures for the daughter products. The smaller isotopic fractionation effects associated with microbial biodegradation of PCE and TCE relative to the daughter products make obtaining convincing field evidence for intrinsic biodegradation based on changes in the $\delta^{13}C$ values of PCE and TCE more of a challenge. Taking this approach will be essential at many sites however since the large fractionation effects associated with the biodegradation daughter products cis-DCE, VC, and ethene only appear when the bulk of the PCE and TCE has been degraded. At many sites such as Dover AFB where significant PCE and TCE still exist and the large isotopic variation in the daughter products has not yet appeared, evaluating the extent of biodegradation will be dependent on interpretation of the smaller fractionation effects associated with the primary contaminants, PCE and TCE. As this study shows, this can be successfully done. Even at a complex site such as Area 6, Dover AFB, with multiple source areas and with the complicating factor of mixing between different contaminant sources, the observed variation in $\delta^{13}C$ values of PCE and TCE can be used to help constrain hypotheses concerning source zones and to definitively identify zones of biodegradation. While estimates of the relative extent of biodegradation between different wells can be obtained, the ultimate goal for this approach should be obtaining a higher level of quantification by increasing the existing database on specific fractionation factors associated with different microbial dechlorinating populations and by integrating the stable isotope constraints into groundwater transport models.

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