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Distinguishing Abiotic and Biotic Transformation of Tetrachloroethylene and Trichloroethylene by Stable Carbon Isotope Fractionation

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Significant carbon isotope fractionation was observed during FeS-mediated reductive dechlorination of tetrachloroethylene (PCE) and trichloroethylene (TCE). Bulk enrichment factors (ϵ_{bulk}) for PCE were -30.2 ± 4.3 ‰ (pH 7), -29.54 ± 0.83 ‰ (pH 8), and -24.6 ± 1.1 ‰ (pH 9). For TCE, ϵ_{bulk} values were -33.4 ± 1.5 ‰ (pH 8) and -27.9 ± 1.3 ‰ (pH 9). A smaller magnitude of carbon isotope fractionation resulted from microbial reductive dechlorination by two isolated pure cultures (*Desulfuromonas michiganensis* strain BB1 (BB1) and *Sulfurospirillum multivorans* (Sm)) and a bacterial consortium (BioDechlor INOCULUM (BDI)). The ϵ_{bulk} values for biological PCE microbial dechlorination were -1.39 ± 0.21 ‰ (BB1), -1.33 ± 0.13 ‰ (Sm), and -7.12 ± 0.72 ‰ (BDI), while those for TCE were -4.07 ± 0.48 ‰ (BB1), -12.8 ± 1.6 ‰ (Sm), and -15.27 ± 0.79 ‰ (BDI). Reactions were investigated by calculation of the apparent kinetic isotope effect for carbon ($AKIE_C$), and the results suggest that differences in isotope fractionation for abiotic and microbial dechlorination resulted from the differences in rate-limiting steps during the dechlorination reaction. Measurement of more negative ϵ_{bulk} values at sites contaminated with PCE and TCE may suggest the occurrence of abiotic reductive dechlorination by FeS.

Introduction

Both mineral- and microorganism-mediated reductive dechlorination likely contribute to the natural attenuation of tetrachloroethylene (PCE) and trichloroethylene (TCE) at contaminated sites (*1*−*14*). While biological transformations may proceed more rapidly than abiotic transformations under optimum conditions, rates of abiotic transformation represent at least the minimum rates of degradation in the field and therefore should be quantified (*15*). Microbial dechlorination via hydrogenolysis frequently results in the problematic accumulation of toxic intermediates (e.g., *cis*-1,2-dichloroethylene (*cis*-DCE) and vinyl chloride (VC)) (*16*). Abiotic dechlorination via reductive β-elimination, on the other hand, leads mainly to the environmentally benign degradation product, acetylene (*1*, *17*−*19*). It is therefore desirable to be able to determine which of these two processes is occurring at contaminated sites.

Stable carbon isotope analysis is a new tool to assess the fate of PCE and TCE in contaminated groundwaters (*19*−*32*). Because the rate constant for cleavage of a chemical bond containing ^{12}C is greater than that for an otherwise equivalent bond containing ^{13}C, reactions for which bond cleavage is the rate-limiting step can result in the enrichment of the heavier isotope (^{13}C) in the remaining parent compound (*30*). The magnitude of isotope fractionation can be described by the bulk enrichment factor, ϵ_{bulk}, derived from the Rayleigh model (*33*). Previously reported ϵ_{bulk} values for abiotic PCE reductive dechlorination (in ‰) include −15.5 to −5.7 for Peerless and Connelly irons (*19*), −16.5 to −15.8 for Vitamin B_{12} at pH 8.8 (*27*), and −14.7 for FeS at pH 7.3 (*29*). For TCE, reported ϵ_{bulk} values for abiotic dechlorination (in ‰) include −10.1 for zerovalent iron filings (*26*), −16.7 for cast and autoclaved electrolytic iron (*25*), −13.9 to −7.5 for Peerless and Connelly irons (*19*), −17.2 to −16.6 for Vitamin B_{12} at pH 8.8 (*27*), and −9.6 at pH 7.3 for FeS (*29*).

ϵ_{bulk} values for microbial reductive dechlorination of PCE and TCE are generally smaller in magnitude (less negative) than those for abiotic reductants, especially for PCE. In his dissertation, Zwank (*29*) concluded that differences in ϵ_{bulk} values could be used to distinguish abiotic and biotic reductive dechlorination of PCE, but not TCE, in model sulfate-reducing systems. Reported ϵ_{bulk} values (in ‰) for PCE microbial reductive dechlorination include -1.02 ± 0.06 (*29*) and -0.42 ± 0.08 (*31*) for *Sulfurospirillum multivorans* (Sm), and −2, −5.5 to −2.7, and −5.18 for microcosms from a PCE-contaminated site (*21*), mixed consortia (*24*), and a pure culture (*31*), respectively. For TCE microbial reductive dechlorination, ϵ_{bulk} values (in ‰) include -12.6 ± 0.5 (*29*) and -16.4 ± 1.5 (*32*) for Sm and −4, −6.6 to −2.5, −7.1, −13.8, and −3.3 to −16 for microcosms from a PCE-contaminated site (*21*), microbial consortia (*22*−*24*), and two pure cultures (*32*), respectively.

Microbial enzyme-catalyzed generally reactions involve a sequence of steps (*29*, *31*, *34*−*35*)

$$S_{out} \underset{(1)}{\rightleftharpoons} S_{in} + E \underset{(2)}{\rightleftharpoons} ES_{in} \underset{(3)}{\longrightarrow} EP_{in} \underset{(4)}{\rightleftharpoons} E + P_{in} \underset{(5)}{\rightleftharpoons} P_{out} \quad (1)$$

where the numbers refer to (1) transport of the substrate (S) from outside (S_{out}) to inside (S_{in}) the cell, (2) formation of the enzyme (E)−substrate complex, (3) bond cleavage and formation of enzyme−product (P) complex, (4) dissociation of enzyme−product complex, and (5) transport of the product from inside (P_{in}) to outside (P_{out}) the cell. Similar schemes involving mass transport of solutes to a mineral surface (step 1), surface complex formation (step 2), electron transfer (step 3), surface complex dissociation (step 4), and mass transport of solutes away from a mineral surface (step 5) have also been proposed for abiotic redox reactions (e.g., *36*); thus eq 1 could apply to abiotic reactions as well. Step 3 is the only step in either scheme involving bond cleavage, and consequently, only step 3 can lead to isotope fractionation (*31*). Step 3 could, however, consist of a series of elementary reaction steps related to bond cleavage, some of which, for example, reduction of a reactive metal center in a dehalogenase enzyme, do not involve C−Cl cleavage. If such a substep were rate limiting, then no isotope fractionation would be observed. In addition, if steps 1, 2, 4, or 5 were rate limiting, little or no isotope fractionation would occur.

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Nijenhuis et al. (*31*) observed an increase in isotope fractionation with a decrease in cell integrity during reductive dechlorination of PCE by Sm and *Desulfitobacterium* sp. Strain PCE-S, which suggests that transport of PCE into the cell (e.g., step 1 in eq 1) is the rate-limiting step in dechlorination by these bacteria.

The hypothesis of our research was that the greater extent of isotope fractionation generally observed for abiotic versus biotic reductive dechlorination of PCE and TCE is the result of rate control by the bond cleavage step (step 3, eq 1) for abiotic reactions and rate control or partial rate control by non-fractionating steps for microbial reactions. This suggestion is reasonable considering the very slow rates of mineral-mediated transformation of PCE and TCE (*1*−*3*, *5*, *8*−*10*, *12*−*14*), which would make rate control by mass transport or surface complexation unlikely. Our objectives were both to test this hypothesis and to evaluate whether stable carbon isotope fractionation could be used to help distinguish between abiotic and microbial reductive dechlorination in the field. To test our hypothesis, we studied both abiotic and microbial reductive dechlorination and performed kinetic and isotope analysis in well-defined model systems. Since preliminary experiments with a variety of mineral species (iron sulfide (FeS), magnetite, pyrite, hydroxysulfate green rust, and goethite treated with HS^- or Fe^{+2}) showed that FeS was the most reactive mineral in abiotic PCE and TCE dechlorination, we chose FeS as a model abiotic system. We then compared results to several bacterial systems including those converting PCE and TCE to *cis*-DCE and a consortium converting PCE and TCE to ethylene. To our knowledge, this is the first paper to provide an explanation for differences in ϵ_{bulk} values for abiotic and microbial reductive dechlorination of PCE and TCE.

Experimental Section

The sources of all chemical reagents and analytical methods used to quantify reactants, products, and isotope values are given in the Supporting Information.

Procedure for Abiotic Experiments. Batch kinetic experiments were conducted at pH 7, 8, and 9 in 5 mL glass ampules containing 10 g/L FeS, 30 μM PCE or TCE, and either HEPES (pH 7 and 8) or CHES (pH 9) buffers (50 mM). In each ampule, the aqueous phase volume was 6.5 mL, and the gas-phase volume was approximately 1.25 mL. Ampules were prepared in an anaerobic chamber containing approximately 96% N_2 and 4% H_2, with a catalytic O_2 removal system (Coy Products, Grass Lake, MI). After preparation, ampules were temporarily covered with polyvinylidene chloride film (Saran Wrap) that was secured with a short piece of plastic tubing (*37*), then taken out of the chamber, and spiked with PCE or TCE stock solution prepared in N_2-sparged methanol. Ampules were then immediately sealed using a methane/oxygen flame while they were kept anaerobic with the Saran Wrap cover; they were then placed in a constant-temperature chamber at 25 °C in the dark on a rocking platform shaker (Labquake, Cole Parmer Instrument Company). At regular intervals, ampules were centrifuged, broken open, and sampled.

Procedure for Biotic Experiments. One stimulated mixed culture, BioDechlor INNOCULUM (BDI), and two isolated pure cultures, Sm and *Desulfuromonas michiganesis* strain BB1 (BB1), were kindly provided by Prof. Frank E. Loeffler at the Georgia Institute of Technology. (Throughout this paper we use the classification *Sulfurospirillum multivorans* (Sm) and not *Dehalosprillum multivorans* (*38*)). BDI is an enriched microbial consortium containing several strains of *Dehalococcoides* (*39*). Sm was isolated from activated sludge not previously exposed to chlorinated ethylenes (*40*−*41*). BB1 was isolated from unpolluted river sediment (*42*).

All culture microcosms were prepared in 1 L Pyrex bottles modified by a glassblower (G. Finkenbeiner Inc., Waltham, MA) to accommodate a septum stopper (Bellco Biotechnology). A reduced anaerobic basal salts medium (BS medium) was prepared according to ref *42*. After the medium was boiled and cooled, the pH was adjusted to 7.2 with 2.52 g/L $NaHCO_3$ under a stream of N_2/CO_2 (80%/20%). A vitamin solution, trace metals (*43*), 0.2 mM L-cysteine, and 0.5 mM Na_2S were added from sterile anaerobic solutions. The electron donors were (all 5 mM) lactate (BDI), acetate (BB1), and pyruvate (Sm). Cultures were inoculated using a 1:50 dilution ratio. Serum bottle microcosms were sealed with sterilized Teflon-lined rubber stoppers (West Pharmaceutical Services) and aluminum seals. Initial concentrations of PCE and TCE in the microcosm experiments were approximately 117 and 108 μM, respectively. Microcosms were prepared in duplicate and incubated in the dark at room temperature. All microcosm manipulations were performed under a stream of sterile N_2/CO_2 gas.

Calculation of ϵ_{bulk} Values. Standard normalized isotope ratios ($^{13}C/^{12}C$ or R) were expressed as $\delta^{13}C$, that is,

$$\delta^{13}C = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample} - \left(\frac{^{13}C}{^{12}C}\right)_{std}}{\left(\frac{^{13}C}{^{12}C}\right)_{std}} \times 1000\ (‰) \qquad (2)$$

The isotopic composition of the parent compound (PCE or TCE) was related to extent of transformation using the Rayleigh model (*33*)

$$R_p = R_{p,o} f^{(\epsilon_{bulk}/1000)} \qquad (3)$$

where R_p is the isotope ratio of the parent compound at any time, $R_{p,0}$ is its isotopic ratio at time zero, f is the fraction of parent compound remaining at a given time (i.e., C/C_0), and ϵ_{bulk} is the bulk enrichment factor. ϵ_{bulk} values were calculated by nonlinear regression using experimentally measured values of $\delta^{13}C$ and f.

Results and Discussion

Abiotic Reductive Dechlorination and Isotope Fractionation. Figure 1 shows that PCE and TCE were degraded following pseudo-first-order kinetics at pH 7, 8, and 9 (PCE) and pH 8 and 9 (TCE) in the presence of FeS. Surface area-normalized pseudo-first-order rate constants (k_{SA} values), adjusted to be equal to those that would be measured in a zero-headspace system (*44*), are reported in Table 1. For TCE at pH 7, degradation was too slow to calculate a rate constant, so no value of k_{SA} is reported in Table 1, and no line showing a pseudo-first-order fit is shown in Figure 1. Dechlorination of PCE and TCE by FeS was strongly pH dependent with faster rates at higher pH values, in agreement with previously reported results (*9*).

Although trends with respect to pH were similar, rate constants for TCE degradation by FeS were considerably lower than previously reported values at similar pH (*9*). This may be the result of use of different pH buffers (tris-(hydroxymethyl)aminomethane (Tris) in ref *9* versus HEPES (pH 7 and 8) or CHES (pH 9) in this study). The addition of 0.01 M Tris to a magnetite/CCl_4 slurry increased the rate constant for CCl_4 reductive dechlorination by a factor of 3 at pH 8.9, compared to the case when no pH buffer was present (*45*). Differences in the surface properties of the FeS samples used in this study versus those in ref *9* could also be responsible for differences in rate constants.

The pH also affected isotope fractionation for PCE and TCE transformation by FeS, quantified by the difference in ϵ_{bulk} values (Table 1) and illustrated by the change in $\delta^{13}C$

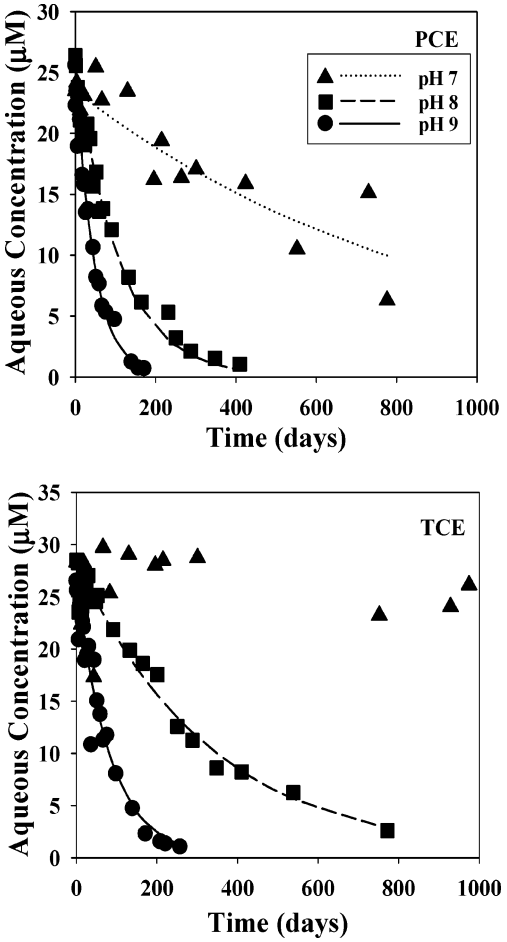

FIGURE 1. Abiotic reductive degradation of PCE and TCE in the presence of FeS at different pH values. Lines represent a pseudo-first-order model fit.

with fraction remaining (Figure 2). The magnitude of isotope fractionation decreased (i.e., ϵ_{bulk} values became less negative) with increasing pH for both PCE and TCE. Acid/conjugate pairs such as $\equiv FeOH_2^+/\equiv FeOH$ (*46*) and $\equiv Fe^{III}OFe^{II+}/\equiv Fe^{III}$-$OFe^{II}OH^0$ (*47*−*49*) have been proposed to exist at reactive mineral surfaces. As discussed in greater detail elsewhere (*30*, *50*), the susceptibility of a bond containing a particular isotope to cleavage (and therefore fractionation) depends in part on its molecular vibrations in the transition state. Assuming the transition state consists of an activated complex between the mineral surface and PCE or TCE, pH-dependent changes in the chemical composition of the mineral surface could affect the transition state structure, molecular vibrations, and isotope fractionation. By "transition state structure" we mean the lengths and angles of partially broken and partially formed bonds in the transition state.

Our ϵ_{bulk} value for PCE dechlorination by FeS at pH 7 (−30.2 ± 4.3 ‰) is quite different than that measured by Zwank (*29*) at an initial pH of 7.3 (−14.7 ‰). This difference may be the result of the presence of 4 mM dissolved Fe(II) (added as $FeCl_2$) in Zwank's experiments. The addition of dissolved Fe(II) would increase non-sulfide Fe(II) at the FeS surface, both weakly bound (i.e., $MgCl_2$ extractable) and strongly bound (i.e., 0.5 N HCl extractable) (*51*), which might have influenced ϵ_{bulk} values. The different ϵ_{bulk} values could also be caused by the presence of HEPES buffer in our experiments, compared to Zwank's unbuffered experiments. Since TCE dechlorination by FeS at pH 7 did not proceed to a great enough extent to calculate an ϵ_{bulk} value (Figure 1), we cannot fairly compare our results to Zwank's results for TCE obtained pH 7.3 (−9.6 ‰) (*29*). Our values at pH 8 and 9, are, however, significantly more negative, perhaps for the reasons described above.

Zwank (*29*) found more isotope fractionation for PCE versus TCE dechlorination by FeS, which he attributed to different transition state compositions for PCE and TCE. Our experiments showed the opposite trend at pH 8 and 9 (Table 1). ϵ_{bulk} values could not be compared at pH 7 because the TCE reaction proceeded too slowly at this pH in our experiments to measure an ϵ_{bulk} value.

Biotic Reductive Dechlorination and Isotope Fractionation. Plots of C/C_0 versus time for microbial transformation of PCE and TCE are shown in Figure 3. Microbial dechlorination took place solely by hydrogenolysis, as evidenced by the good mass recovery (generally >80%) of hydrogenolysis products (Figure 3). ϵ_{bulk} values for microbial dechlorination are reported in Table 1 and plots of $\delta^{13}C$ versus f are shown in Figure 2. Our measured ϵ_{bulk} values for dechlorination by Sm are similar to most previously reported values (*29*, *31*) but are less negative than the value of −16.4 ± 1.5 ‰ reported by Lee et al. for TCE (*32*).

Table 1 and Figure 2 also show a greater magnitude of isotope fractionation for microbial TCE dechlorination compared to PCE dechlorination for all cultures, as found in previous studies (*24*, *29*). For Sm, this trend was explained by different values of "commitment to catalysis" for PCE and TCE (*29*). The commitment to catalysis equals the rate of step 3 divided by the reverse of step 2 (eq 1). Different commitments to catalysis would reflect different affinities of PCE and TCE for the dehalogenase enzyme.

Comparison of Abiotic Versus Biotic Microcosms. Isotope fractionation of PCE and TCE during abiotic transformation was consistently stronger than fractionation during biotic transformation (Table 1 and Figure 2). To understand why, we calculated additional isotope parameters for the abiotic and biotic systems. While ϵ_{bulk} values represent the overall isotope fractionation for an entire molecule, the kinetic isotope effect for carbon (KIE_C) equals the rate constant for cleavage of a $^{12}C-Cl$ bond divided by that for a $^{13}C-Cl$ bond (i.e., $^{12}k/^{13}k$) and thus represents isotope effects resulting from C−Cl bond cleavage. We calculated values of the "apparent" kinetic isotope effect for carbon ($AKIE_C$) from ϵ_{bulk} values using the approach described in Elsner et al. (*30*) and Zwank et al. (*52*). This approach considers two factors: (*1*) the presence of C atoms at positions in a molecule that are nonreactive (i.e., C atoms with no potential for bond cleavage) and (*2*) the presence of different isotopes at more than one equally reactive position in a molecule (intramolecular competition). These two factors can result in dilution or enhancement of the $AKIE_C$ and can be accounted for using the following equation (*52*):

$$\frac{1}{AKIE_C} = \frac{z\, n\, \epsilon_{bulk}}{1000\, x} + 1 \qquad (4)$$

where n is the number of C atoms in the molecule, x is the number of C atoms with the potential for bond cleavage, and z is the number of C atoms having equal reactivity.

We then compared our calculated $AKIE_C$ values with theoretical KIE_C values for C−Cl bond cleavage to determine whether the rate-limiting processes in the overall transformation reaction involved bond cleavage (step 3 in eq 1) or other steps. With the assumption that bond cleavage is rate limiting, the $AKIE_C$ and KIE_C values should be the same (*30*). We used a KIE_C value of 1.03, estimated by semiclassical Streitwieser limits (*50*) and assuming 50% bond cleavage in the transition state (*30*, *53*). The term "semiclassical" means that this parameter was calculated using a combination of classical and quantum mechanical assumptions (*50*). While the extent of bond cleavage in the transition state is not

TABLE 1. Rate Constants, ϵ_{bulk} Values, and Apparent Kinetic Isotope Effects for Carbon ($AKIE_C$ Values)

	conditions	k_{SA}[a] (L m^{-2} d^{-1})	ϵ_{bulk} (‰)[b]	mechanism of reductive β-elimination[c]	n[c]	x[c]	z[c]	$AKIE_C$[a]
PCE	FeS, pH 7	$(6.3 \pm 1.6) \times 10^{-5}$	-30.2 ± 4.3	1	2	2	2	1.0644 ± 0.0097
				2	2	2	1	1.0312 ± 0.0045
	FeS, pH 8	$(5.30 \pm 0.51) \times 10^{-4}$	-29.54 ± 0.83	1	2	2	2	1.0628 ± 0.0019
				2	2	2	1	1.03044 ± 000088
	FeS, pH 9	$(1.21 \pm 0.12) \times 10^{-3}$	-24.6 ± 1.1	1	2	2	2	1.0517 ± 0.0025
				2	2	2	1	1.0252 ± 0.0012
	BB1	NA[d]	-1.39 ± 0.21	NA[d]	2	2	2	1.00278 ± 0.00043
	Sm	NA	-1.33 ± 0.13	NA	2	2	2	1.00266 ± 0.00027
	BDI	NA	-7.12 ± 0.72	NA	2	2	2	1.0145 ± 0.0015
TCE	FeS, pH 8	$(1.61 \pm 0.19) \times 10^{-4}$	-33.4 ± 1.5	1	2	1	1	1.0715 ± 0.0034
				2	2	2	1	1.0345 ± 0.0016
	FeS, pH 9	$(6.40 \pm 0.81) \times 10^{-4}$	-27.9 ± 1.3	1	2	1	1	1.0592 ± 0.0030
				2	2	2	1	1.0287 ± 0.0014
	BB1	NA[d]	-4.07 ± 0.48	NA[d]	2	1	1	1.0082 ± 0.0010
	Sm	NA	-12.8 ± 1.6	NA	2	1	1	1.0262 ± 0.0034
	BDI	NA	-15.27 ± 0.79	NA	2	1	1	1.0315 ± 0.0017

[a] Uncertainties are 95% confidence intervals calculated by propagation of error. For k_{SA} values, we assumed that the major error was from determination of rate constants because errors from measurement of surface area and mass loading were typically less than 5%. [b] Uncertainties are 95% confidence intervals calculated from nonlinear regression. [c] See text discussion. [d] NA means not applicable.

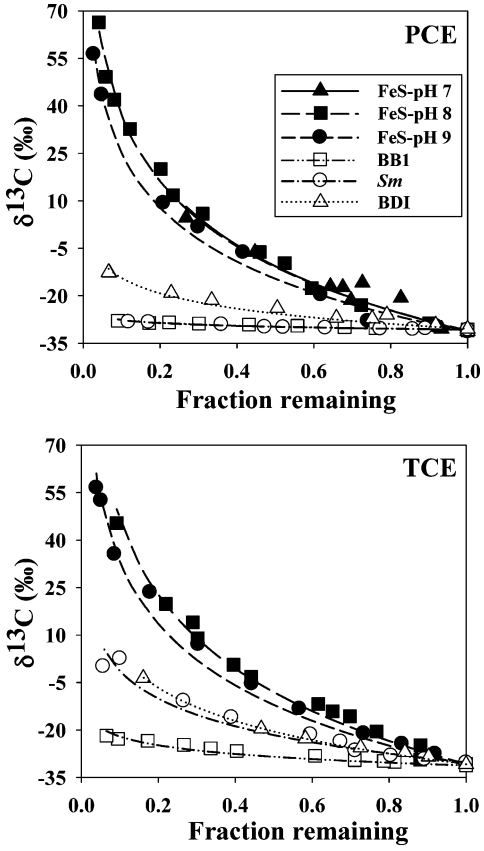

FIGURE 2. Isotope fractionation during the reductive dechlorination of PCE and TCE by abiotic and biotic microcosms. Lines represent a Rayleigh model fit.

known, this value provides a consistent basis for comparison of our biotic and abiotic experiments. The lower and upper limits of the KIE_C using semiclassical Streitwieser limits are 1.00 (for 0% bond cleavage in the transition state) and ~1.057 (for 100% bond cleavage in the transition state) (*53*).

The most commonly proposed mechanism for hydrogenolysis involves a rate-limiting carbon−halogen bond cleavage step that takes place concurrent with electron transfer (*54*). For PCE hydrogenolysis, $x = 2$ and $z = 2$ because both C atoms are identical chemically and therefore have equal potential for bond cleavage. For TCE hydrogenolysis, $x = 1$ and $z = 1$ because the lengths and therefore strengths of the C−Cl bonds vary with C position (*55*−*56*), and thus the two C atoms have different potentials for cleavage. Additional evidence for the unequal reactivity of the two C atoms in TCE is the preponderance of *cis*-1,2-DCE and not 1,1-DCE, as the TCE hydrogenolysis product.

While biotic reductive dechlorination took place entirely by hydrogenolysis, abiotic reductive dechlorination of PCE and TCE occurred by both hydrogenolysis and reductive β-elimination, as evidenced by detection of the products of both reaction pathways, specifically *cis*-DCE and, for PCE, TCE (hydrogenolysis) and acetylene (reductive β-elimination). PCE and TCE reductive β-elimination yields acetylene via short-lived dichloro- (for PCE) and chloroacetylene (for TCE) intermediates (*17*). Because of problems quantifying acetylene, we do not report or illustrate our reductive β-elimination product yields, but a previous study found that the major pathway for PCE and TCE transformation by FeS was reductive β-elimination and not hydrogenolysis (*5*). Consistent with this, we calculated TCE hydrogenolysis yields of 12.7% at pH 8 and 2.6% at pH 9 using the method of Fennelly and Roberts (*57*), confirming that hydrogenolysis was a minor pathway for TCE. We could not quantify PCE hydrogenolysis yields without acetylene concentration values because the TCE from PCE hydrogenolysis can transform to acetylene via reductive β-elimination (*5*).

Two mechanisms are possible for reductive β-elimination, each with different x and z values for eq 4. (Regardless of pathway or mechanism, $n = 2$ for both PCE and TCE.) One of us previously proposed that, as for hydrogenolysis, reductive β-elimination of PCE and TCE by FeS involves an initial rate-limiting C−Cl cleavage step (*5*). We refer to this as "mechanism 1" below. Another mechanism involving simultaneous carbon−halogen bond cleavage and C−C bond formation, referred to below as "mechanism 2", is also well-known for nucleophiles like sulfide (*58*−*62*). For reductive β-elimination by mechanism 1, x and z for PCE and TCE are identical to those for hydrogenolysis. For mechanism 2, $x =$ 2 for PCE and TCE because both C−Cl bonds are broken in

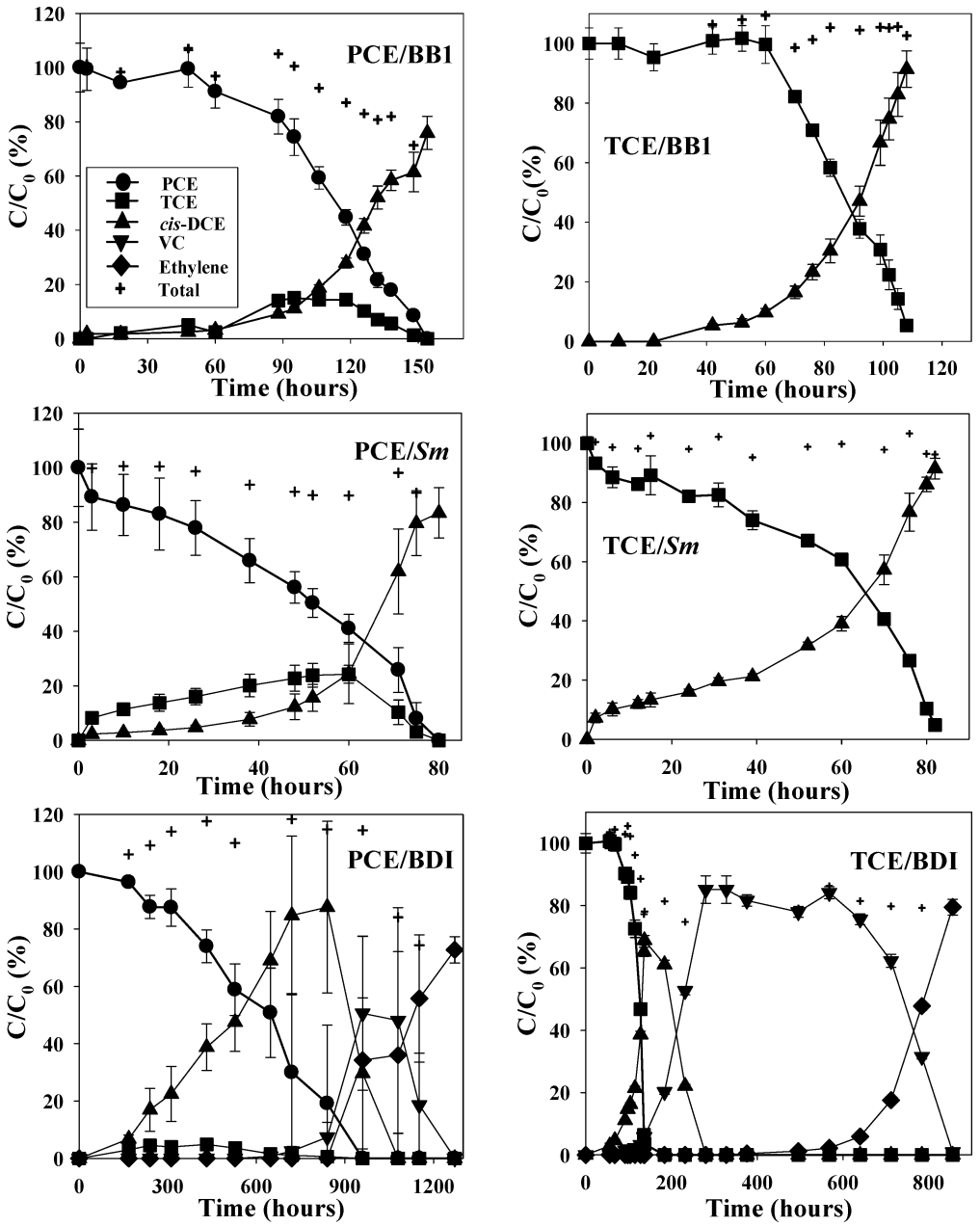

FIGURE 3. Microbial reductive degradation of PCE by (A) BB1, (B) Sm, and (C) BDI and TCE by (D) BB1, (E) Sm, and (F) BDI. Error bars represent 95% confidence intervals for mean values from three microcosms.

the rate-limiting step, and $z = 1$, since there is no intramolecular competition (*52*). $AKIE_C$ values for reductive β-elimination were calculated first assuming mechanism 1 and then mechanism 2. All values of n, x, and z and the resulting values of $AKIE_C$ are summarized in Table 1.

We first observed that the $AKIE_C$ values for microbial PCE and TCE reductive dechlorination were generally less than the theoretical KIE_C of ~1.03 for C−Cl bond cleavage. This could be caused by rate limitation by one or more nonfractionating processes (i.e., steps 1, 2, 4, and 5 in eq 1), rather than C−Cl bond cleavage. On the other hand, TCE dechlorination by Sm and PCE and TCE dechlorination by BDI had $AKIE_C$ values closer to the theoretical value than did the other cultures (Table 1), suggesting that the rate of PCE or TCE dechlorination by these cultures is more strongly influenced by the rate of C−Cl bond cleavage (eq 1, step 3).

Table 1 shows that most $AKIE_C$ values for abiotic PCE and TCE reductive β-elimination calculated assuming mechanism 1 are near the top or outside the theoretical range of KIE_C values for C−Cl bond cleavage calculated using semiclassical Streitwieser limits (*53*) (i.e., 1.00−1.057). While $AKIE_C$ values calculated assuming mechanism 2 are within this range (Table 1), comparison of these values to the theoretical KIE_C for a single C−Cl bond cleavage is not valid because other bond breaking and formation steps are also involved in a concerted mechanism, like mechanism 2. Specifically, the strong driving force for formation of an additional C−C bond (i.e., the triple bond in the reactive chloro- and dichloroacetylene intermediates that yield acetylene) likely influences the theoretical KIE_C for mechanism 2 because atomic mass (i.e., ^{12}C or ^{13}C) affects the driving force for bond formation as well as bond cleavage.

Despite uncertainty about the mechanism of reductive β-elimination of PCE and TCE by FeS, the $AKIE_C$ values for PCE and TCE transformation by this pathway probably lie between those calculated assuming mechanisms 1 and 2. It

is noteworthy that these values are generally significantly larger than those for microbial dechlorination of PCE and TCE (Table 1), suggesting that fractionating processes such as C−Cl bond cleavage and not mass transport steps like diffusion and surface complex formation, limit the rate of abiotic reductive dechlorination. This is consistent with the slow rate of FeS-mediated PCE and TCE transformation (half-lives on the order of months (Figure 1)), for which mass transport and reactive complex formation are likely to be much faster than bond cleavage and associated electron transfer.

Environmental Significance. These experiments showed that ϵ_{bulk} values were more negative for PCE and TCE reductive dechlorination by FeS than by three dechlorinating cultures isolated from different locations. Thus, measurement of more negative ϵ_{bulk} values at contaminated sites undergoing natural attenuation may indicate the occurrence of abiotic reductive dechlorination of PCE and TCE by FeS. Although the distribution of reaction products in groundwater could in theory also distinguish between biotic (hydrogenolysis) and abiotic (predominantly reductive β-elimination) transformation of PCE and TCE, complete mass recovery of all reaction products is not typical at field sites, perhaps because of biodegradation of infrequently detected reaction products such as acetylene (e.g., *63*).

Another application of these results involves use of stable C isotope fractionation to distinguish PCE and TCE reductive dechlorination from non-fractionating processes such as advection, dispersion, and sorption (*64*−*65*). The use of an erroneously small (i.e., less negative) ϵ_{bulk} value for this purpose would result in overestimation of contaminant degradation. Our reported ϵ_{bulk} values for abiotic PCE and TCE degradation are more negative than those for previously studied systems and should be considered when evaluating the performance of remediation technologies (including natural attenuation) that may involve abiotic transformation of PCE and TCE by FeS.

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Supporting Information Available

Sources of chemical reagents and description of analytical methods and isotope measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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