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Insights into Enzyme Kinetics of Chloroethane Biodegradation Using Compound Specific Stable Isotopes

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While compound specific isotope analysis (CSIA) has been used extensively to investigate remediation of chlorinated ethenes, to date considerably less information is available on its applicability to chlorinated ethanes. In this study, biodegradation of 1,1,1-trichloroethane (1,1,1-TCA) and 1,1-dichloroethane (1,1-DCA) was carried out by a *Dehalobacter*-containing mixed culture. Carbon isotope fractionation factors (ε) measured during whole cell degradation demonstrated that values for 1,1,1-TCA and 1,1-DCA (−1.8‰ and −10.5‰, respectively) were significantly smaller than values reported for abiotic reductive dechlorination of these same compounds. Similar results were found in experiments degrading these two priority pollutants by cell free extracts (CFE) where values of −0.8‰ and −7.9‰, respectively, were observed. For 1,1,1-TCA in particular, the large kinetic isotope effect expected for cleavage of a C−Cl bond was almost completely masked during biodegradation by both whole cells and CFE. Comparison to previous studies demonstrates that these patterns of isotopic fractionation are not attributable to transport effects across the cell membrane, as had been seen for other compounds such as PCE. In contrast these results reflect significant differences in the kinetics of the enzymes catalyzing chlorinated ethane degradation.

Introduction

1,1,1-Trichloroethane (1,1,1-TCA) and 1,1-Dichloroethane (1,1-DCA) are chlorinated aliphatic hydrocarbons used as industrial solvents and degreasing agents, and are produced as intermediates in the production of vinyl chloride. 1,1,1-TCA and 1,1-DCA are common groundwater contaminants, found in at least 23% and 19%, respectively, of the active Superfund sites designated by the U.S. Environmental Protection Agency (search of the database in February 2010). Under anaerobic conditions, metabolic and cometabolic reductive dechlorination of these compounds have been observed under methanogenic (*1*−*3*) and sulfate-reducing conditions (*4*). During reductive dechlorination, chlorine atoms are sequentially removed and replaced with hydrogen atoms, so that transformation proceeds from 1,1,1-TCA to 1,1-DCA to chloroethane (CA) analogous to reductive dechlorination of their chlorinated ethene counterparts trichloroethene (TCE), and cis-dichloroethene (cDCE), to vinyl chloride (VC) and finally to ethene. Bioremediation has become a cost-effective strategy for cleanup of sites contaminated with chlorinated ethenes (*5*). For 1,1,1-TCA and 1,1-DCA however, optimum conditions for dechlorination are less well understood, partly because of the complex inhibitory effects of mixtures of chlorinated ethenes and ethanes. Since over 8% of sites on the U.S. Environmental Protection Agency Superfund list are cocontaminated with both 1,1,1-TCA and TCE, resolving the basis for such inhibition is the focus of investigation by several groups (*6*−*9*). Recently, Grostern et al. (2009) (*10*) reported that the most pronounced inhibition of 1,1,1-TCA and 1,1-DCA was observed in the presence of chlorinated ethenes in cell-free extract experiments - suggesting inhibition directly affects the efficiency of the reductive dehalogenase enzyme. This study reports for the first time the carbon isotope fractionation factors during biodegradation of 1,1,1-TCA and 1,1-DCA. Subsequent studies will then be able to use this base of information to determine whether different fractionation factors are observed when chlorinated ethene and ethane contaminant mixtures undergo biodegradation.

Compound Specific Isotope Analysis (CSIA) is increasingly applied as a tool for assessing biodegradation of organic contaminants in groundwater (*11*). Laboratory-derived ε values have been successfully applied to monitor the occurrence of biodegradation in the field (*11*), but also as a novel means of estimating the extent of biodegradation and quantifying biodegradation rates (*12, 13*) for a variety of organic contaminants including chlorinated ethenes, chlorinated ethanes, petroleum hydrocarbons, and fuel additives such as methyl tert-butyl ether (MTBE). Since fundamentally ε values are a function of the bonds broken during transformation, CSIA has also become an established approach for differentiating between different reaction mechanisms when the role of multiple potential biodegradation pathways are in question (*14, 15*). Understanding the magnitude and variability in the isotopic enrichment factors (ε values) involved in biodegradation is critical to these applications. In particular, considerable recent effort has been focused on determining not just the controls on ε due to differences in degrading microorganisms (to a first approximation controlled primarily by the microbial transformation pathway the organisms use), but on the role of kinetics and enzyme efficiency in controlling observed fractionation effects (*16*−*19*).

While carbon isotopic fractionation has been extensively studied for reductive dechlorination of chlorinated ethenes (*11*), and for 1,2-dichloroethane (1,2-DCA) (*15, 16, 20*−*23*), to our knowledge, no published studies have reported isotopic fractionation factors for microbial reductive dechlorination of 1,1,1-TCA and 1,1-DCA. The first objective of the present study as noted was to determine carbon isotope fractionation factors for reductive dechlorination of these two priority compounds by the *Dehalobacter*-containing mixed culture described by Grostern and Edwards (2006) (*24*). In addition, those experiments were repeated with cell-free extracts of the same culture in order to investigate the differences between observed fractionation during whole cell degradation versus degradation when the substrates were in direct contact with the enzyme. Finally fractionation effects during biodegradation were compared to carbon isotope fractionation for abiotic reductive dechlorination of 1,1,1-TCA by

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Cr(II), Fe(0), and Cu-plated iron (*25*) and for abiotic reductive elimination of Cl from 1,1-DCA on Zn^0 (*26*).

Materials and Methods

Detailed materials and methods for all experiments are provided in the Supporting Information. In brief, for whole cell degradation experiments, ten experimental bottles contained 10 mL of defined mineral medium after (*27*) plus 40 mL of inoculum from the parent microbial enrichment culture were each prepared in 125 mL glass bottles sealed with threaded Teflon-lined Mininert caps and maintained in an anaerobic glovebox under a $CO_2/H_2/N_2$ atmosphere (10%/10%/80%) at room temperature. Four control bottles contained 50 mL of defined mineral medium. All bottles were amended with methanol, ethanol, acetate, and lactate (MEAL) as electron donors at five times each the electron equivalents required for dechlorination. Five experimental bottles and two control bottles were amended with 0.002 mL of neat 1,1,1-TCA (TCA-1, TCA-2, TCA-3, TCA-4, TCA-5), and similarly five experimental bottles and two controls were amended with 0.002 mL of neat 1,1-DCA (DCA-1, DCA-2, DCA-3, DCA-4, DCA-5) - to produce an aqueous concentration of 50 mg/L prior to the isotope experiments.

For biodegradation by cell-free extracts (CFEs) (see preparation details in the SI) assays were prepared in 16 mL glass bottles as described above. Each bottle contained 1.7 mL of assay buffer (100 mM Tris-Cl, pH 7.5, 2 mM methyl viologen, 2 mM titanium citrate) and was amended with either 1,1,1-TCA (0.005 mL of an 8 mg/mL stock solution) (two experimental bottles; CFE-TCA-1, CFE-TCA-2) or 1,1-DCA (0.008 mL of a 5 mg/mL stock solution) (three experimental bottles; CFE-DCA-1, CFE-DCA-2, CFE-DCA-3) for a starting aqueous concentration of approximately 20 mg/L in each bottle. After equilibration, a small amount (0.3 mL) of CFE was added to each experimental bottle to ensure a slow biodegradation rate on the order of days rather than hours (see details in the SI) - thus ensuring sufficient time to collect a large number of $\delta^{13}C$ values for the degradation. In addition, two 1,1,1-TCA and two 1,1-DCA control bottles were prepared (buffer and heat-killed controls), with the CFE heated at 80 °C for 15 min prior to use in the heat-treated controls.

Details of compositional and isotopic analysis are provided in the SI.

Results

For all experiments, the concentration of 1,1,1-TCA (or 1,1-DCA) in controls remained within error of the starting concentration, and the $\delta^{13}C$ values of control bottles remained within analytical uncertainty of $\delta^{13}C_0$ throughout the experiments.

Reductive Dechlorination in Whole Cell Cultures. Concentration versus time plots for all experiments are shown in Figure S-1A-D. Complete degradation of 1,1,1-TCA occurred in all five replicate bottles in 75 to <300 h (~3−12 days) (see Figure S-1A). As degradation of 1,1,1-TCA proceeded, $\delta^{13}C$ values of 1,1,1-TCA became progressively enriched in ^{13}C, consistent with the preferential rate of reaction for ^{12}C. Experimental data for all five bottles fit a Rayleigh model with r^2 values >0.9 for each bottle (Table 1). Enrichment factors (ε) calculated for 1,1,1-TCA in all five experimental bottles were the same within 95% confidence intervals (Table 1) and hence are all included in Figure 1A from which an overall combined ε value of −1.8‰ ± 0.3‰ (95% confidence interval) is calculated for 1,1,1-TCA reductive dechlorination.

Degradation of 1,1-DCA occurred in all five replicate bottles in 200−300 h (8−12 days) (Figure S-1B). For one replicate, analysis was discontinued at 40% degradation at >500 h. As for 1,1,1-TCA, during degradation of 1,1-DCA, all five replicates had calculated ε values that were within a 95% confidence interval of each other and fit a Rayleigh model with r^2 values all >0.9 (Table 1). Based on this, a combined ε value of −10.5‰ ± 0.6‰ (95% confidence interval) is calculated for 1,1-DCA reductive dechlorination by whole cells and shown in Figure 1A.

TABLE 1. Measured ε for All Experiments and All Replicate Bottles[a]

		ε		
compound	replicate no.	(‰)	r^2	95% CI
whole cell 1,1,1-TCA	1	−1.5	0.95	0.6
	2	−1.5	0.99	0.2
	3	−1.8	0.98	0.4
	4	−1.7	0.97	0.3
	5	−1.8	0.97	0.4
	all	−1.8	0.95	0.3
whole cell 1,1-DCA	1	−10.0	0.99	0.6
	2	−9.8	0.94	1.7
	3	−11.2	0.90	1.3
	4	−10.9	0.99	0.8
	5	−11.4	0.99	0.9
	all	−10.5	0.98	0.6
cell-free extract 1,1,1-TCA	1	−0.8	0.84	0.4
	2	−0.8	0.73	0.6
	all	−0.8	0.81	0.3
cell-free extract 1,1-DCA	1	−8.6	0.93	2.2
	2	−7.9	0.98	1.0
	3	−7.8	0.94	1.9
	all	−7.9	0.94	0.9

[a] Goodness of fit to the Rayleigh Model is described by r^2; confidence intervals (CI) are calculated from standard deviation of regression slope.

The whole cell experiments clearly show a distinct difference in carbon isotope fractionation for biodegradation of 1,1,1-TCA compared to biodegradation of 1,1-DCA. As has been observed previously for chlorinated ethenes, the less chlorinated compound has a significantly larger fractionation (ε value of −10.5 ‰ for 1,1-DCA versus −1.8 ‰ for 1,1,1-TCA). The observed fractionation and ε values for replicates of 1,1,1,-TCA were identical within uncertainty (Table 1) despite significant differences in the rate of degradation between replicates (Figure S-1A). The same is true for the replicates of 1,1-DCA (Figure S-1B). This is unlikely to be the case if for instance mass transfer limitations were a factor in these experiments.

Degradation in Cell-Free Extracts. Complete degradation of 1,1,1-TCA occurred in ~70 h (3 days) in both replicate bottles (Figure S-1C). The replicates showed complete agreement with a combined ε value of −0.8‰ ± 0.3‰ and a fit to the Rayleigh model of $r^2 = 0.8$ (Figure 1B, Table 1). For CFE experiments of 1,1-DCA, complete degradation took place in ~170 h (~7 days) (Figure S-1D), and all three replicate bottles (CFE-DCA-1, CFE-DCA-2, CFE-DCA-3) also showed a good fit to the Rayleigh model with r^2 values >0.9 and a combined ε value of −7.9 ± 0.9‰ (95% confidence interval) (Table 1). All isotope data for CFE experiments for both 1,1,1-TCA and 1,1-DCA are shown in Figure 1B.

As for the whole cell experiments, in the CFE experiments, roughly complete degradation (80−100%) occurred on the scale of 3−7 days (Figure S-1C,D). Protocol tests routinely carried out in the laboratory have demonstrated that equilibration across the air−water interface occurs in <5 h — indicating that the experimental results are not attributable to mass transfer effects.

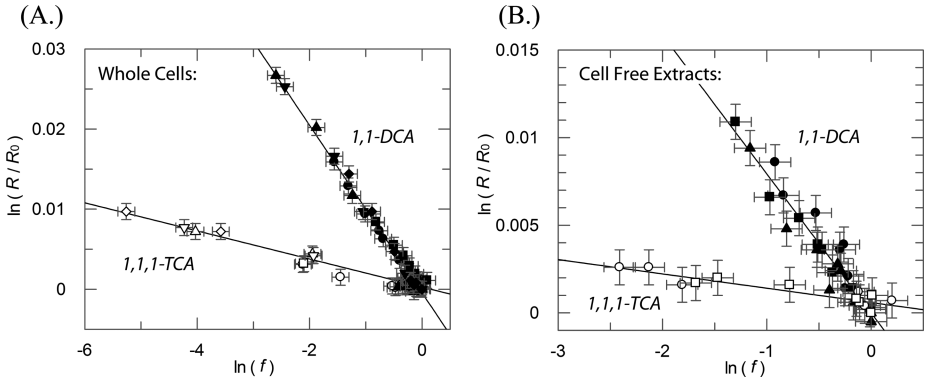

FIGURE 1. Isotopic results for degradation of 1,1,1-TCA (open symbols) and 1,1-DCA (solid symbols) by (A.) whole cells and by (B.) cell-free extracts, respectively. Values of ln(R/R_0) are based on measured $\delta^{13}C$ values and f = fraction of substrate remaining as described in the Supporting Information. Error bars are based on total uncertainty of ±0.5‰ in $\delta^{13}C$ and ±7% on C/C_0. In each panel, the solid line represents the least squared regression through the 1,1,1-TCA (open symbols) and 1,1-DCA (solid symbols) data and the calculated Rayleigh model based on all experimental data combined for each compound. Calculated ε values, confidence intervals, and r^2 fits to the Rayleigh model are in Table 1.

TABLE 2. Measured ε Values and Calculated AKIE Values of Degraded 1,1,1-TCA and 1,1-DCA by Abiotic Processes and by Biodegradation by Whole Cells and Cell-Free Extracts (CFE)[d]

	ε			AKIE		
compound	abiotic	biotic whole cell	biotic CFE	abiotic[c]	biotic whole cell	biotic CFE
1,1,1-TCA	−15.8 to −13.6[a]	−1.8 ± 0.3	−0.8 ± 0.3	1.030 ± 0.002[a]	1.0036 ± 0.0006	1.0016 ± 0.0006
1,1-DCA	−17.9 to −21.2[b]	−10.5 ± 0.6	−7.9 ± 0.9	1.040 ± 0.002[b]	1.021 ± 0.002	1.016 ± 0.002

[a] Values obtained for experiments of abiotic reductive cleavage of one C−Cl bond by Cr(II), Fe^0, and Cu and Fe mixtures (*25*). [b] Values obtained for experiments of abiotic reductive cleavage of one C−Cl bond by Zn^0 (*26*). [c] Both estimates of abiotic fractionation for cleavage of a single C−Cl bond agree with theoretical intrinsic KIE estimates from the literature (*25, 26, 28*). [d] Values from this study reported as ε ± 95% CI (confidence interval) (see Table 1).

To interpret the results of this study in the context of kinetic isotope effects, measured enrichment factors (ε) first have to be converted into kinetic isotope values ($^{12}k/^{13}k$). Since 1,1,1-TCA and 1,1-DCA contain two carbon atoms and due to the low natural abundance of ^{13}C, generally only one of them at most will be ^{13}C, the kinetic isotope effect in this case is defined as proposed in Elsner et al. (2005) (*28*)

$$^{12}k/^{13}k = 1/(1 + (\varepsilon \text{ x } 2)/1000) \quad (1)$$

Discussion

As has been observed for degradation of MTBE (*29*) and chlorinated ethenes (*19, 25, 30, 31*), microbial reductive dechlorination of 1,1,1-TCA and 1,1-DCA demonstrates a significantly smaller carbon isotope fractionation effect than abiotically mediated degradation of these compounds even though cleavage of a single C−Cl bond is involved for both chloroethanes. The ε value measured for 1,1-DCA biodegradation in whole cell cultures is approximately half of the estimated value for abiotic reductive dechlorination, and this difference is even more pronounced for 1,1,1-TCA (Table 2). Dechlorination of 1,1,1-TCA in whole cell cultures shows an exceptionally suppressed enrichment factor of −1.8 ± 0.3‰, compared to published values for abiotic reductive dechlorination that range from −15.8‰ to −13.6‰ (*25*). As shown in Table 1, based on the correlation coefficients, all experimental data fit a Rayleigh model, which means that within each whole cell degradation experiment the extent of fractionation observed is reproducible. The differences observed in isotopic fractionation between the abiotic and biotic experiments are substantial and may be caused by masking of the intrinsic isotope effect associated with C−Cl bond cleavage (*32, 33*).

Recent literature has discussed a variety of processes that might account for such masking effects, including transport across the cell membranes (*17, 34, 35*) - an obvious potential hypothesis given its well-known role in depressing observed carbon isotope effects during photosynthesis (*36*). Nijenhuis et al. (2005) (*19*) investigated the role of membrane transport for PCE dechlorination using a variety of pure cultures, cell free extracts (CFE), and pure enzymes. They observed significantly larger fractionation for pure enzyme experiments and cell-free extracts compared to whole cell degradation, which suggested that for PCE at least, transport across the membrane may indeed affect the fractionation and mask the ε value observed during biodegradation by whole cells. In contrast, no resolvable difference between fractionation by whole cell cultures and by cell free extracts was identified in similar studies conducted on TCE (*37*), 1,2-DCA, and three chlorinated propanes (*16*), suggesting that for these compounds, transport across the cell membrane does not change the observed fractionation.

To determine if membrane transport is involved in suppressing the intrinsic kinetic isotope effect in this case, cell free extract experiments were performed on 1,1,1-TCA and 1,1-DCA. The results were significantly different than those observed for PCE by Nijenhuis et al. (2005) (*19*). For the two replicates for CFE-TCA, measured ε values were not resolvably different from the ε value for degradation by whole cells (Table 1). While there may be subtle differences between fractionation during degradation by whole cells (combined ε −1.8‰ ± 0.3‰) and by CFE (combined ε −0.8‰ ± 0.3‰) for 1,1,1-TCA, the important fact remains that in both cases the measured ε values are still very small compared to measured ε values for abiotic experiments and compared to the theoretical intrinsic KIE value of 1.03−1.06 (*25, 28*). These findings eliminate membrane transport as the underlying source of the

masking effect. Similarly, the CFE results for 1,1-DCA do not support the membrane transport masking hypothesis. Unlike results for PCE (*19*), for 1,1-DCA, ε values measured for CFE (-7.9 ± 0.9 ‰) are actually significantly smaller than for degradation by whole cells (-10.5 ± 0.6 ‰) - suggesting a mechanism other than membrane transport is controlling the observed fractionation patterns.

As noted, the measured abiotic ε values for 1,1,1-TCA and 1,1-DCA of 1.03 to 1.04, respectively (Table 2), fall within the range of theoretical intrinsic carbon KIE values from the literature for cleavage of a single C−Cl bond (*25, 28*). Since the significantly smaller ε values measured in both whole cell and CFE experiments compared to abiotic degradation cannot be attributed to membrane transport, it suggests that masking of the isotope effect is occurring within the enzyme itself and is influenced by the enzyme kinetics. A simplified example of this scenario is well-described by Northrup et al. (1981) (*33*)

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \tag{2}$$

where E represents the enzyme, S represents the substrate, ES represents the enzyme−substrate complex, and P represents the products. k_1 and k_{-1} are the first order rate constants for the formation and dissociation of the enzyme−substrate complex, respectively, and k_2 is the rate constant for the irreversible, chemical transformation of the substrate. If we assume that there are negligible isotope effects on k_1 and k_{-1}, the relationship between the apparent kinetic isotope effect (AKIE), the intrinsic isotope effect (KIE), and the individual rate constants during an enzymatic reaction can be related by the following equation (*32, 33*)

$$\mathrm{AKIE} = \frac{\mathrm{KIE} + C}{1 + C} \tag{3}$$

where AKIE is the apparent kinetic isotope effect derived from the ε measured during biodegradation experiments and KIE is the fully expressed intrinsic isotope effect originating from the irreversible transformation step of the substrate (k_2). The constant C is referred to as the commitment factor and is equal to the rate constant ratio k_2/k_{-1} that describes the tendency of the enzyme−substrate complex to undergo transformation of the substrate (k_2) rather than to dissociate into free enzyme and substrate (k_{-1}). Equation 3 indicates that the magnitude of isotopic fractionation depends on the steps prior to and including the first irreversible transformation of the substrate. In a case where the efficiency of the enzymatic reaction is limited (large k_{-1}) relative to the chemical transformation of the substrate (small k_2) and $k_{-1} \gg k_2$, the C-value becomes negligibly small so that the AKIE is equal in magnitude to the intrinsic KIE (*32, 33, 38*). In contrast, in an extremely efficient enzymatic reaction ($k_2 \gg k_{-1}$), a larger C-value is obtained, and the observed kinetic isotope effect (AKIE) is significantly reduced (masked) compared to the intrinsic KIE.

The results of these experiments (Table 2) can be understood in the context of this model. Whole cell biodegradation for both 1,1,1-TCA and 1,1-DCA shows significant masking of the observed isotope effect (smaller AKIE) compared to the intrinsic KIE observed in the abiotic degradation experiments. For 1,1,1-TCA in particular, the isotope effect is almost completely masked, with an AKIE of 1.0036 suggesting a highly efficient enzymatic conversion step (i.e., large k_2 versus k_{-1}). In contrast, while whole cell degradation of 1,1-DCA shows significant masking of the AKIE (1.021) compared to the intrinsic KIE in the abiotic experiments (1.040), the masking effect depressed the value by only roughly 50% compared to the order of magnitude effect that was seen for 1,1,1-TCA. This is highlighted by the difference in C-values for each reaction. In 1,1-DCA the C-value is unity, while for 1,1,1-TCA the value is significantly greater than unity. C-values near unity typically suggest that the enzyme catalyzed process involves two or more steps that have similar energy barriers, instead of a single rate-limiting step. In the abiotic mechanism, a single rate-limiting step is associated with the highest energy barrier (e.g., carbon-chlorine bond cleavage) and leads to a large intrinsic kinetic isotope effect. In the analogous enzyme catalyzed mechanism, an alternative chemical pathway is provided that has multiple lower energy barriers of similar height that leads to a reduced or masked apparent kinetic isotope effect and a C-value that approaches unity (ref *32* and references therein). For 1,1,1-TCA, the C-value is significantly greater than unity, which suggests that the abiotic rate-limiting step is no longer kinetically significant in the enzyme catalyzed pathway. This latter case suggests 1,1,1-TCA degradation involves an enzymatic system with optimal catalytic efficiency.

Grostern et al. (2009) (*10*) recently reported that the enzyme affinity (K_m) for 1,1,1-TCA dechlorination in cell-free extracts for this culture (45 ± 18 μM) is an order of magnitude greater than the enzyme affinity for 1,1-DCA dechlorination ($K_m = 413 \pm 64$ μM) and that degradation of these two compounds is performed by two distinct reductive dehalogenases. This is strongly supported by the distinctly different C-values found in the current study that suggest unique catalytic mechanisms exist for each contaminant. This type of behavior has been previously reported in enzyme catalyzed decarboxylation reactions that involve cofactors, where the rate-limiting step changes from carbon−carbon bond cleavage in the uncatalyzed pathway to bond formation between the substrate and cofactor in the enzymic pathway producing C-values significantly greater than unity (*32, 33*).

There are several significant implications of these results. First, the results clearly indicate that one cannot assume that isotopic fractionation for degradation by CFE will always be larger than for whole cell experiments. Depending on the nature of the rate-limiting steps contributing to the AKIE in addition to the intrinsic bond cleavage, CFE experiments may show fractionation that is either larger or smaller than for whole cell degradation experiments. While in practice abiotic fractionation factors are often considered to be closest to the intrinsic KIEs, it is important to also note that additional rate-limiting steps can be present in purely chemical (abiotic) reactions that can also lead to masking of the KIE. This has been recently demonstrated for chemical experiments on the decarboxylation reaction mechanism (*39, 40*).

Second, the use of kinetic isotope effects is an essential tool for the identification of different reaction pathways in these systems. They can reveal key information related to the chemical mechanisms and rate-limiting processes involved in these complex degradation pathways; however, this information cannot be obtained from a single ε-value. We have shown that by comparing multiple ε-values and kinetic isotope effects for abiotic, biotic, and cell free extract experiments we can obtain a much clearer picture of the mechanisms and pathways involved in these systems. Moreover, we have shown that comparing C-values can provide further insight into the mechanisms involved at the level of the enzyme.

Finally, for field applications, this study demonstrates in the case of biodegradation of 1,1,1-TCA, one of the most extreme cases of masking of intrinsic KIE effects yet published for reductive dechlorination. Since enzyme kinetics play such a significant role in the expression of the KIE for both 1,1,1-TCA and 1,1-DCA, clearly careful work will be needed to determine the range of expression of fractionation factors for the chlorinated ethanes and the controls exerted by cocontaminants with inhibitory effects such as the chlori-

nated ethenes. Given the number of Superfund sites where both types of chlorinated solvents occur in mixed plumes, this question is an important target for future research, in particular before CSIA can be used to quantify biodegradation of these compounds.

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Supporting Information Available

An expanded Materials and Methods, all concentrations versus time plots, and fundamental definitions and equations for isotopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Gander, J. W.; Parkin, G. F.; Scherer, M. M. Kinetics of 1,1,1-trichloroethane transformation by iron sulfide and a methanogenic consortium. *Environ. Sci. Technol.* **2002**, *36* (21), 4540–4546.

(2) de Best, J. H.; Hage, A.; Doddema, H. J.; Janssen, D. B.; Harder, W. Complete transformation of 1,1,1-trichloroethane to chloroethane by a methanogenic mixed population. *Appl. Environ. Microb.* **1999**, *51* (2), 277–283.

(3) Vogel, T. M.; McCarty, P. L. Abiotic and Biotic Transformations of 1,1,1-Trichloroethane under Methanogenic Conditions. *Environ. Sci. Technol.* **1987**, *21* (12), 1208–1213.

(4) de Best, J. H.; Salminen, E.; Doddema, H. J.; Janssen, D. B.; Harder, W. Transformation of carbon tetrachloride under sulfate reducing conditions. *Biodegradation* **1997**, *8* (6), 429–436.

(5) Aulenta, F.; Majone, M.; Tandoi, V. Enhanced anaerobic bioremediation of chlorinated solvents: environmental factors influencing microbial activity and their relevance under field conditions. *J. Chem. Technol. Biot.* **2006**, *81* (9), 1463–1474.

(6) Yu, S. H.; Dolan, M. E.; Semprini, L. Kinetics and inhibition of reductive dechlorination of chlorinated ethylenes by two different mixed cultures. *Environ. Sci. Technol.* **2005**, *39* (1), 195–205.

(7) Yu, S.; Semprini, L. Kinetics and modeling of reductive dechlorination at high PCE and TCE concentrations. *Biotechnol. Bioeng.* **2004**, *88* (4), 451–464.

(8) Cupples, A. M.; Spormann, A. M.; McCarty, P. L. Vinyl chloride and cis-dichloroethene dechlorination kinetics and microorganism growth under substrate limiting conditions. *Environ. Sci. Technol.* **2004**, *38* (4), 1102–1107.

(9) Sun, B. L.; Griffin, B. M.; Ayala-del-Rio, H. L.; Hashsham, S. A.; Tiedje, J. M. Microbial dehalorespiration with 1,1,1-trichloroethane. *Science* **2002**, *298* (5595), 1023–1025.

(10) Grostern, A.; Chan, W. W. M.; Edwards, E. A. 1,1,1-Trichloroethane and 1,1-Dichloroethane reductive dechlorination kinetics and co-contaminant effects in a *Dehalobacter*-containing mixed culture. *Environ. Sci. Technol.* **2009**, *43*, 6799–6807.

(11) Hunkeler, D.; Meckenstock, R. U.; Sherwood Lollar, B.; Schmidt, T. C.; Wilsont, J. T. *A Guide for Assessing Biodegradation and Source Identification of Organic Groundwater Contaminants using Compound Specific Isotope Analysis (CSIA)*; EPA 600/R-08/148; United States Environmental Protection Agency: Ada, OK, Dec. 2008, 2008; p 68.

(12) Morrill, P.; Lacrampe-Couloume, G.; Slater, G. F.; Sleep, B. E.; Edwards, E. A.; McMaster, M. L.; Major, D. W.; Sherwood Lollar, B. Quantifying chlorinated ethene degradation during reductive dechlorination at Kelly AFB using stable carbon isotopes. *J. Contam. Hydrol.* **2005**, *76*, 279–293.

(13) Sherwood, Lollar, B.; Slater, G. F.; Sleep, B.; Witt, M.; Klecka, G. M.; Harkness, M.; Spivack, J. Stable carbon isotope evidence for intrinsic bioremediation of tetrachloroethene and trichloroethene at Area 6, Dover Air Force Base. *Environ. Sci. Technol.* **2001**, *35*, 261–269.

(14) Zwank, L.; Berg, M.; Elsner, M.; Schmidt, T. C.; R.P., S.; Haderlein, S. B. New evaluation scheme for two-dimensional isotope analysis to decipher biodegradation processes: Application to groundwater contamination by MTBE. *Environ. Sci. Technol.* **2005**, *39* (4), 1018–1029.

(15) Hirschorn, S. K.; Dinglasan, M. J.; Elsner, M.; Mancini, S. A.; Lacrampe-Couloume, G.; Edwards, E. A.; Sherwood Lollar, B. Pathway dependant isotopic fractionation during aerobic biodegradation of 1,2-dichloroethane. *Environ. Sci. Technol.* **2004**, *38*, 4775–4781.

(16) Abe, Y.; Zopfi, J.; Hunkeler, D. Effect of molecule size on carbon isotope fractionation during biodegradation of chlorinated alkanes by Xanthobacter autotrophicus GJ10. *Isot. Environ. Health Stud.* **2009**, *45* (1), 18–26.

(17) Lee, P. K. H.; Conrad, M. E.; Alvarez-Cohen, L. Stable carbon isotope fractionation of chlorethenes by dehalorespiring isolates. *Environ. Sci. Technol.* **2007**, *41*, 4277–4285.

(18) Mancini, S. A.; Hirschorn, S. K.; Elsner, M.; Lacrampe-Couloume, G.; Sleep, B.; Edwards, E. A.; Sherwood Lollar, B. Effects of trace element concentration on enzyme controlled stable isotope fractionation during aerobic Biodegradation of toluene. *Environ. Sci. Technol.* **2006**, *40*, 7675–7681.

(19) Nijenhuis, I.; Andert, J.; Beck, K.; Kastner, M.; Diekert, G.; Richnow, H. H. Stable isotope fractionation of tetrachloroethene during reductive dechlorination by *Sulfurospirillum multivorans* and *Desulfitobacterium sp*. Strain PCE-S and abiotic reactions with cyanocobalamin. *Appl. Environ. Microb.* **2005**, *71* (7), 3413–3419.

(20) Hirschorn, S. K.; Dinglasan, M. J.; Edwards, E. A.; Lacrampe-Couloume, G.; Sherwood Lollar, B. Compound specific isotope analysis as a natural reaction probe to determine biodegradation mechanisms. *Environ. Microbiol.* **2007**, *9*, 1982–1998.

(21) Hirschorn, S. K.; Grostern, A.; Lacrampe-Couloume, G.; Edwards, E. A.; MacKinnon, L.; Repta, C.; Major, D. W.; Sherwood Lollar, B. Quantification of Biodegradation of chlorinated hydrocarbons: Added value via stable carbon isotope analysis. *J. Contam. Hydrol.* **2007**, *94*, 249–260.

(22) Hunkeler, D.; Aravena, R.; Berry-Spark, K. L.; Cox, E. Assessment of degradation pathways in an aquifer with mixed chlorinated hydrocarbon contamination using stable isotope analysis. *Environ. Sci. Technol.* **2005**, *39* (16), 5975–5981.

(23) Hunkeler, D.; Aravena, R. Evidence of substantial carbon isotope fractionation among substrate, inorganic carbon, and biomass during aerobic mineralization of 1,2-dichloroethane by *Xanthobacter autotrophicus*. *Appl. Environ. Microb.* **2000**, *66* (11), 4870–4876.

(24) Grostern, A.; Edwards, E. A. A 1,1,1-trichloroethane-degrading anaerobic mixed microbial culture enhances biotransformation of mixtures of chlorinated ethenes and ethanes. *Appl. Environ. Microb.* **2006**, *72* (12), 7849–7856.

(25) Elsner, M.; Cwiertny, D. M.; Roberts, A. L.; Sherwood Lollar, B. 1,1,2,2-Tetrachloroethane reactions with OH^-, Cr(II), granular iron and a copper-iron bimetal: Insights from product formation and asociated carbon isotope fractionation. *Environ. Sci. Technol.* **2007**, *41*, 4111–4117.

(26) VanStone, N. A.; Elsner, M.; Lacrampe-Couloume, G.; Mabury, S. A.; Sherwood Lollar, B. Potential for identifying abiotic chloroalkane degradation mechanisms using stable carbon isotopes. *Environ. Sci. Technol.* **2008**, *42*, 126–132.

(27) Edwards, E. A.; Wills, L. E.; Reinhard, M.; Grbic-Galic, D. Anaerobic degradation of toluene and xylene by aquifer microorganisms under sulfate-reducing conditions. *Appl. Environ. Microb.* **1992**, *58* (3), 794–800.

(28) Elsner, M.; Zwank, L.; Hunkeler, D.; Schwarzenbach, R. P. A new concept linking observable stable isotope fractionation to transformation pathways of organic pollutants. *Environ. Sci. Technol.* **2005**, *39*, 6896–6916.

(29) Elsner, M.; McKelvie, J.; Couloume, G. L.; Lollar, B. S. Insight into methyl tert-butyl ether (MTBE) stable isotope fractionation from abiotic reference experiments. *Environ. Sci. Technol.* **2007**, *41* (16), 5693–5700.

(30) Slater, G. F.; Lesage, S.; Brown, S.; Sherwood Lollar, B. Stable carbon isotope fractionation during reductive dechlorination of PCE and TCE by Vitamin B12. *Ground Water Monit. Rem.* **2003**, *23*, 59–67.

(31) Liang, X.; Dong, Y.; Kuder, T.; Krumholz, L. R.; Philp, R. P.; Butler, E. C. Distinguishing abiotic and biotic transformation of PCE and TCE by stable carbon isotope fractionation. *Environ. Sci. Technol.* **2007**, *41*, 7094–7100.

(32) Cleland, W. W. Northrop, D. B. O'Leary, M. H. *Isotope effects on enzyme-catalyzed reactions: [proceedings of the Sixth Annual Harry Steenbock Symposium, held in Madison, Wisconsin, on June 4 and 5, 1976]*; University Park Press: Baltimore, 1977; p xi, 303p.

(33) Northrop, D. B. Intrinsic isotope effects in enzyme-catalyzed reactions. In *Enzyme Mechanism from isotope effects*; Cook, P. F., Ed.; CRC Press: 1981.

(34) Morrill, P. L.; Sleep, B. E.; Slater, G. F.; Edwards, E. A.; Sherwood Lollar, B. Evaluation of isotopic enrichment factors for the Biodegradation of chlorinated ethenes using a parameter estimation model: Towards an improved quantification of Biodegradation. *Environ. Sci. Technol.* **2006**, *40*, 3886–3892.

(35) Slater, G. F.; Sherwood Lollar, B.; Sleep, B. E.; Edwards, E. A. Variability in carbon isotopic fractionation during Biodegradation of chlorinated ethenes: Implications for field applications. *Environ. Sci. Technol.* **2001**, *35* (5), 901–907.

(36) O'Leary, M. H. Carbon isotopes in photosynthesis. *Bioscience* **1988**, *38*, 328–336.

(37) Cichocka, D.; Siegert, M.; Imfeld, G.; Andert, J.; Beck, K.; Diekert, G.; Richnow, H. H.; Nijenhuis, I. Factors controlling the carbon isotope fractionation of tetra- and trichloroethene during reductive dechlorination by *Sulfurospirillum ssp.*, and *Desulfitobacterium sp.* strain PCE-S. *FEMS Microbiol. Ecol.* **2007**, *62*, 98–107.

(38) Melander, L.; Saunders, W. H., Jr. *Reaction rates of isotopic molecules*; Wiley: New York, 1980; p 331.

(39) Mundle, S. O. C.; Lacrampe-Couloume, G.; Sherwood Lollar, B.; Kluger, R. Hydrolytic decarboxylation of carboxylic acids and the formation of protonated carbonic acid. *J. Am. Chem. Soc.* **2010**, *132*, 2430–2436.

(40) Mundle, S. O. C.; Rathgeber, S.; Lacrampe-Couloume, G.; Sherwood Lollar, B.; Kluger, R. Internal return of carbon dioxide in decarboxylation: Catalysis of separation and C-12/C-13 kinetic isotope effects. *J. Am. Chem. Soc.* **2009**, *131* (33), 11638–11639.

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