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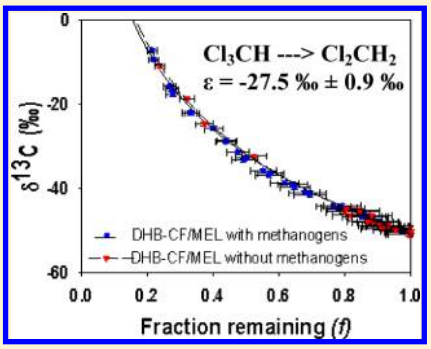
Large Carbon Isotope Fractionation during Biodegradation of Chloroform by *Dehalobacter* Cultures

Calvin C. H. Chan,[†] Scott O. C. Mundle,[†] Thomas Eckert,[†] Xiaoming Liang,[†] Shuiquan Tang,[‡] Georges Lacrampe-Couloume,[†] Elizabeth A. Edwards,[‡] and Barbara Sherwood Lollar[†,*]

[†]Department of Earth Sciences, University of Toronto, Toronto, ON M5S 3B1, Canada

[‡]Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON M5S 3E5, Canada

ABSTRACT: Compound specific isotope analysis (CSIA) has been applied to monitor bioremediation of groundwater contaminants and provide insight into mechanisms of transformation of chlorinated ethanes. To date there is little information on its applicability for chlorinated methanes. Moreover, published enrichment factors (ε) observed during the biotic and abiotic degradation of chlorinated alkanes, such as carbon tetrachloride (CT); 1,1,1-trichloroethane (1,1,1-TCA); and 1,1-dichloroethane (1,1-DCA), range from −26.5‰ to −1.8‰ and illustrate a system where similar C−Cl bonds are cleaved but significantly different isotope enrichment factors are observed. In the current study, biotic degradation of chloroform (CF) to dichloromethane (DCM) was carried out by the *Dehalobacter* containing culture DHB-CF/MEL also shown to degrade 1,1,1-TCA and 1,1-DCA. The carbon isotope enrichment factor (ε) measured during biodegradation of CF was −27.5‰ ± 0.9‰, consistent with the theoretical maximum kinetic isotope effect for C−Cl bond cleavage. Unlike 1,1,1-TCA and 1,1-DCA, reductive dechlorination of CF by the *Dehalobacter*-containing culture shows no evidence of suppression of the intrinsic maximum kinetic isotope effect. Such a large fractionation effect, comparable to those published for *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC) suggests CSIA has significant potential to identify and monitor biodegradation of CF, as well as important implications for recent efforts to fingerprint natural versus anthropogenic sources of CF in soils and groundwater.



INTRODUCTION

Natural attenuation of contaminants can occur via a variety of degradative and nondegradative processes at field sites. Though both degradative and nondegradative processes can decrease dissolved contaminant concentrations, actual transformation to nontoxic end products is desirable.[1] Quantifying the extent of intrinsic degradation relies on a broad range of evidence including microbiological, chemical field measurements, field and laboratory experiments, and modeling. Challenges stem from the inability to obtain accurate mass balance of the contaminant, electron donor and end products in field sites because nondegradative processes, such as volatilization, sorption and transport also contribute to changes in contaminant concentrations.[1]

Compound specific isotope analysis (CSIA) has been shown to be an important approach for directly assessing the extent of bioremediation of chlorinated ethenes, MTBE, and BTEX in the subsurface.[2] As a result of the preferential degradation rate of molecules containing exclusively ^{12}C, a remaining contaminant pool becomes relatively enriched in the heavier ^{13}C isotopes as degradation proceeds. CSIA determines the carbon isotopic signatures by measuring two stable isotopes of ^{12}C and ^{13}C. This ratio is expressed as $\delta^{13}C$ (units of ‰) = $(R_{sample}/R_{standard} - 1) \times 1000$, where R_{sample} is the $^{13}C/^{12}C$ ratio in a given sample and $R_{standard}$ is the $^{13}C/^{12}C$ ratio in the international standard reference material, V-PDB. For many priority organic pollutants, the relationship between isotopic fractionation and extent of degradation can be modeled by the Rayleigh model.[2,3] The Rayleigh model assumes constant isotope fractionation during degradation that is represented by α. The fractionation factor, α relates the isotopic composition of the substrate at a given time, R, to the initial isotopic composition, R_0, and to the fraction of substrate remaining f where $R/R_0 = f^{(\alpha-1)}$. The α value is directly equivalent to the inverse kinetic isotope effect at the reactive site $\alpha = (^{12}k/^{13}k)^{-1}$, and can also be expressed as an enrichment factor, ε, where $\varepsilon = 1000\ (\alpha-1)$. The rate constants ^{12}k and ^{13}k are for bond cleavage involving ^{12}C and ^{13}C carbon isotopes respectively and $^{12}k/^{13}k$ defines the kinetic isotope effect (KIE). Isotope enrichment factors (ε) are, to a first approximation, a function of the bonds broken during degradation and have been shown to be powerful tools for differentiating between reaction mechanisms,[4] pathways[5] and enzyme kinetics in biochemical reactions.[6] However, additional controls on isotope fractionation can cause variation in observed kinetic isotope effects, even for degradation reactions undergoing similar bond cleavages.[6−8] Understanding and establishing ε values of degradative

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processes for specific compounds are the basic knowledge required for applying CSIA to monitor field situations.[2]

While significant research has been done to determine the carbon isotope fractionation effects associated with biotic and abiotic degradation of chlorinated ethenes[2,3,7,9] and chlorinated ethanes,[4−6,10,11] to date chlorinated methanes such as CF have received significantly less attention. Initially used as an anesthetic and a chemical precursor to now banned refrigerants (e.g., HCFC-22), CF is a common contaminant in over 50% of US Environmental Protection Agency's National Priority list sites.[12,13] Chloroform is listed as a possibly carcinogenic substance with high exposure concerns by the International Agency for Research on Cancer[14] and the US EPA maximum contaminant level is 80 μg/L for drinking water.[12] More importantly, the presence of CF is toxic to many microbial processes vital for bioremediation, reductive dechlorination, and detoxification of chlorinated ethenes.[15,16] Bioremediation is often a cost-effective method for cleaning up contaminated sites but CF inhibits chlorinated-ethene degrading cultures when present even at low concentrations.[16,17]

Understanding the chemical mechanisms of CF degradation may allow for improved remediation efforts at contaminated field sites. Studies of CF biodegradation[18−22] have identified some of the degradation products and pathways responsible via radioisotope studies and kinetic analyses that investigated the effect of cyanocobalamin[19] and metallo-porphyrin[23] on CF degradation rates. Lee et al.[22] demonstrated complete dechlorination of CF by organochlorine respiration to DCM and subsequent fermentation of DCM. Like CF, CT degradation occurs under various conditions in both aerobic and anaerobic environments via both biotic and abiotic processes.[24−27] CSIA has been applied to distinguish possible chemical mechanisms responsible for the surface-mediated reductive dehalogenation of CT by Fe-bearing minerals[11,28,29] and some CSIA measurements have been made for CT at field sites.[30] To date, no corresponding work on isotope effects during biodegradation or abiotic degradation of CF has been reported. Nikolausz et al.[31] characterized carbon isotope fractionation during DCM degradation by methylotrophic bacteria under aerobic and NO_3-reducing conditions and showed the same magnitude of fractionation in all cases suggesting similar dehalogenation mechanisms for all DCM-degrading cultures investigated to date.[32]

Recently, Grostern et al.[12] reported a *Dehalobacter*-containing enrichment culture capable of degrading chlorinated ethanes (1,1,1-TCA and 1,1-DCA) by reductive dechlorination and also of transforming CF via chlororespiration to the less toxic dichloromethane (DCM) as a final end-product. Maintained on an electron donor solution referred to as MEL (methanol, ethanol and lactate in a mole ratio of 2:1:1), the culture is referred to as DHB-CF/MEL. Two subcultures were derived from the parent culture DHB-CF/MEL and used in this study. The microbial composition of a methanogen-free CF-degrading subculture was determined from 16S rRNA pyrotag sequencing provided by the Department of Energy Joint Genome Institute (http://genome.jgi.doe.gov/genome-projects/pages/project-status.jsf?projectId=403125) to consist predominantly of *Dehalobacter* (∼∼79%); *Bacteroides* (∼9%) and *Clostridium* (∼7%) − the latter two are fermenting organisms. Derived from the same parent DHB-CF/MEL culture, a methanogen-containing CF subculture also contains *Methanocullaceae* belonging to the order *Methanomicrobiales*. Previous studies on DHB-CF/MEL indicated that 1,1,1-TCA and 1,1-DCA were degraded by two distinct reductive dehalogenases with different kinetic parameters, K_m and V_{max}[33] and different observed carbon isotope fractionation factors.[6] The existence of two distinct reductive dehalogenases has since been further confirmed by sequencing (http://genome.jgi.doe.gov/genome-projects/pages/project-status.jsf?projectId=401933) and biochemical analysis of partially purified proteins. The two enzymes are highly homologous to each other and share an amino acid identity of 95.2%, yet they have distinct chlorinated substrates. One enzyme dechlorinates CF and 1,1,1,-TCA but not 1,1-DCA, while the other degrades only 1,1-DCA and not CF or 1,1,1-TCA.

The first objective of this study was to determine the isotope enrichment factors observed during dechlorination of CF by these *Dehalobacter*-containing subcultures and to compare this to previous results observed during reductive dechlorination of 1,1,1-TCA and 1,1-DCA by the same culture. All these processes occur via cleavage of a single C−Cl bond via reductive dechlorination − a cleavage that has a theoretical kinetic isotope effect (KIE) of ∼1.03.[34] While abiotic degradation of 1,1,1-TCA and 1,1-DCA showed carbon isotope fractionation effects consistent with this large theoretical value, biodegradation of these two compounds by the *Dehalobacter*-containing culture showed substantially smaller fractionation effects − a masking effect known to occur if the enzyme mediating the transformation reaction is highly efficient.[6] In the case of 1,1,1-TCA in particular, fractionation was suppressed by an order of magnitude (KIE of 1.0036) compared to the theoretical KIE. In contrast, for 1,1-DCA only a partial masking effect was observed (KIE of 1.02), consistent with the lower enzyme affinity for the 1,1-DCA degrading enzyme compared to that of the enzyme degrading 1,1,1-TCA.[6] The first objective of this study was to determine carbon isotope fractionation effects during biodegradation of CF by the *Dehalobacter* enzyme that also degrades 1,1,1-TCA, in particular to determine if a masking effect similar to that seen for 1,1,1-TCA is observed.

As a secondary objective, experiments were conducted to characterize carbon isotope fractionation during CF biodegradation by the DHB-CF/MEL subcultures, with and without methanogens. While DHB-CF/MEL is the only published enrichment culture to chlororespire CF, cometabolic transformation of CF can occur, often associated with methanogenesis.[18,35] There was no indication of cometabolic degradation of CF in the methanogen-containing subculture used in this study. Further, methane production in the methanogenic-containing subculture was limited when CF was present, reflecting CF inhibition of methanogenic activity. While it was not anticipated that methanogenic bacteria played a role in the degradation of CF in the subculture, both methanogen-containing and nonmethanogen-containing DHB-CF/MEL subcultures were used in the experiments to determine if the presence/absence of the methanogens had any effect on the observed carbon isotope fractionation effects.

MATERIALS AND METHODS

Experimental Design. Prior to the isotopic experiments, all cultures were maintained with chloroform as the sole carbon and energy source in a defined mineral medium[36] and amended with the electron donor solution MEL. During the isotope experiments, both DHB-CF/MEL subcultures were maintained in 1 L pyrex bottles and fed 0.1 mL of neat CF at regular intervals. The experiment bottles were maintained in an

anaerobic glovebox under a CO_2/ H_2/ N_2 atmosphere (10%/ 10%/ 80%) at room temperature. All experiments were conducted in 160 mL glass serum bottles (Wheaton) sealed with blue butyl stoppers (Bellco Glass, Inc.) after the method of Slater et al.[9] For each experimental bottle 40 mL of fresh media was added along with 4 uL of isotopically characterized CF (−50.5 ± 0.5 ‰), and all bottles were equilibrated overnight. Equilibration of CF between the aqueous and gas phase takes approximately 6 h as determined by laboratory protocol tests.

For all experiments both killed controls and media controls were set up to ensure that the isotopic compositions were not affected by experimental design or sampling procedure. The killed control was prepared with 50 mL of autoclaved media, 10 mL of bacterial culture and 100 μL of HgCl. The media control was set up in an identical fashion to the experimental bottles except that no bacterial culture was added. Similar to the experimental bottles, 4 uL of isotopically characterized in house working standard CF (−50.5 ± 0.5 ‰) was added to each control in 160 mL serum bottles (Wheaton) and capped with the blue butyl stopper (Bellco Glass, Inc.). In the experimental bottles, approximately 10 mL of bacterial culture were added. CF and DCM concentrations and the isotopic composition of CF were measured throughout the biodegradation experiments.

Concentration Analysis. CF and DCM concentration were measured by removing a 300 uL headspace sample from a culture or control bottle using a 500 uL Pressure-Lok gastight syringe (Precision Sampling Corp.) then injecting the sample into Varian 3400 gas chromatograph equipped with a flame ionization detector (FID) and an Agilent J&W GSQ capillary column (30 m × 0.53 mm) after the method of Slater et al.[9] The injector temperature was set at 200 °C and detector at 210 °C. After the injection of a sample, the oven temperature held at 140 °C for 2 min and then ramped to 175 at 3 °C/minute. The temperature was then increased to 210 °C at a rate of 20 °C/minutes and held isothermally at 210 °C for 1 min. Three point external calibration curves were prepared daily. Relative standard deviations for standards using this method were ±0.5%.

Isotope Analysis. Stable carbon isotopes for CF were analyzed by direct headspace after the method of Slater et al.[9] Each analysis was done in duplicate or triplicate. Reproducibility (precision) on replicate analyses is typically 0.1−0.3‰ but total accuracy and reproducibility for $\delta^{13}C$ values is ±0.5‰.[2,37] All analyses are calibrated to in house isotopically characterized reference materials. Accuracy tests and linearity tests are performed daily.[2] Enrichment factors (ε) were determined using a derivation of the Rayleigh isotopic model expressed as $\ln[(\delta_t/1000 + 1)/(\delta_0/1000 + 1)] = (\varepsilon/1000) \times \ln f$, where δ_0 is the initial isotopic composition of the substrate and δ_t is the isotopic composition of the substrate measured at each time point. This is done by plotting ln f versus $\ln[(\delta_t/1000 + 1)/(\delta_0/1000 + 1)]$ and determining the slope of the linear regression (m) which is related to the fractionation factor by $m = (\varepsilon/1000)$.

RESULTS

Controls. CF concentrations in the control bottles were 710 ± 30 μM and 738 ± 20 μM respectively and no change in concentration outside of error (±5%) was observed throughout the experiments. No changes were observed in the $\delta^{13}C$ of CF in the control bottles compared to the initial $\delta^{13}C$ value of the isotopically characterized CF used in all the experiments (−50.5 ± 0.5 ‰) (data not shown).

Reductive Dechlorination of DHB-CF/MEL with methanogens. The DHB-CF/MEL culture with methanogens transformed CF to DCM via reductive dechlorination over 225 h (~ 10 days) (Figure 1A). The degradation rates between the three experimental bottles were similar. As degradation of CF proceeded, $\delta^{13}C$ values for CF remaining became more enriched in ^{13}C, consistent with a preferential rate of reaction for ^{12}C during microbial transformation to DCM. In all culture bottles, a significant enrichment in ^{13}C of the remaining chloroform occurred as biodegradation proceeded, producing values as enriched as −9.4 ‰ at the end of the experiment (Figure 2).

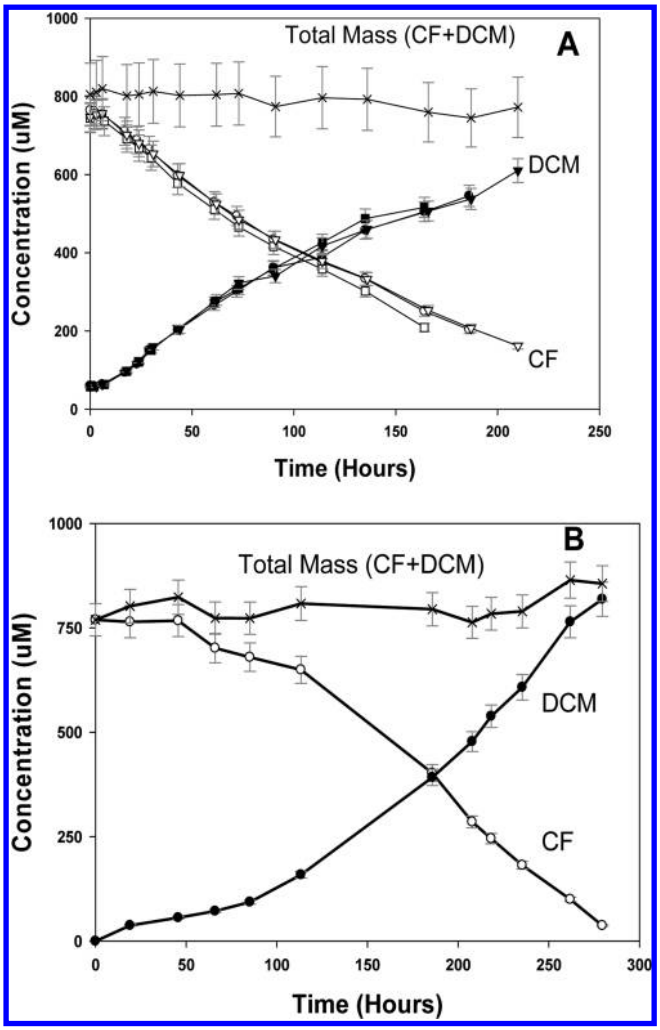

Figure 1. Reductive dechlorination of CF by DHB-CF/MEL cultures (A) CF degradation by DHB-CF/MEL culture with methanogens: concentrations of CF and DCM versus time. (B) CF degradation of DHB-CF/MEL culture without methanogens: concentration of CF and DCM versus time. Error bars represent ±5% error as described in text. Open symbols represent CF concentration. Solid symbols represent DCM concentration. X represents calculated total mass of CF and DCM combined.

Reductive Dechlorination of DHB-CF/MEL without Methanogens. The DHB-CF/MEL culture without methanogens transformed CF via reductive dechlorination over 275 h (~11 days) (Figure 1B). The degradation rate and mass balance in this experiment were similar to those described

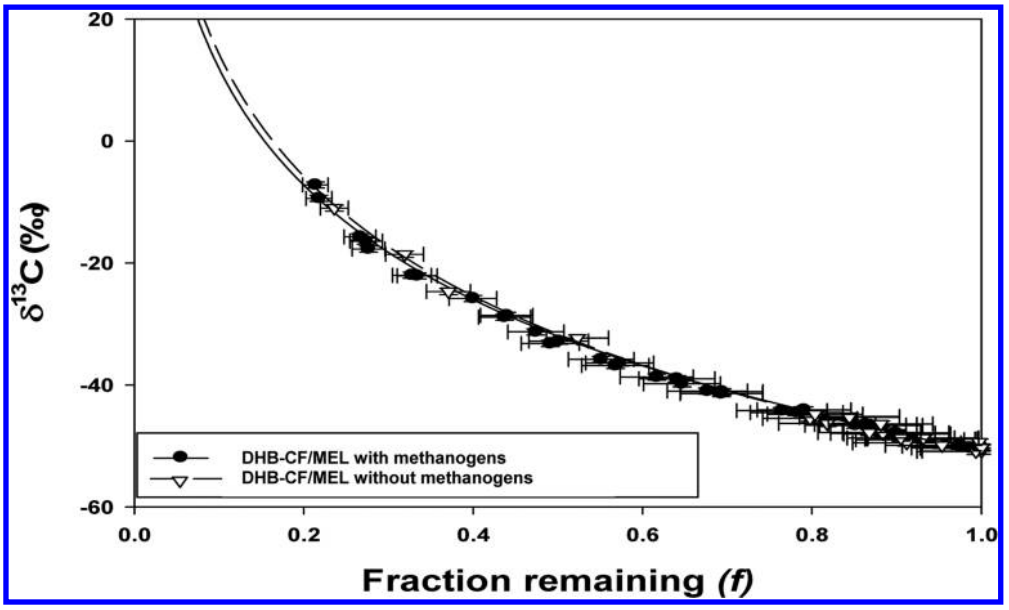

Figure 2. Isotopic results for degradation of CF by DHB-CF/MEL with methanogens and DHB-CF/MEL without methanogens. Values on the *x* axis represent fraction of substrate remaining (error bars are ±7%) and values on *y* axis are δ^{13}C values (error bars are ±0.5 ‰). The solid line represents the Rayleigh model for DHB-CF/MEL with methanogens and the hatched line represents Rayleigh model for DHB-CF/MEL without methanogens. Enrichment factors (ε) are identical within uncertainty (Table 1).

above and at the end of the experiment CF δ^{13}C values were as enriched as −11.0 ‰ (Figure 2).

Carbon Isotope Enrichment Factors. Table 1 lists the enrichment factors (ε) determined for each bottle in experiments using DHB-CF/MEL with and without methanogens as well as the mean ε value for all replicate bottles for each experiment. Experimental data for all bottles fit a Rayleigh model with r^2 values >0.99 indicating an excellent fit between experimental data and the model (Table 1). All three bottles have ε values within 95% confidence intervals of each other resulting in a cumulative ε value for all three bottles of −27.6 ‰ ± 0.9 ‰ (Table 1). While the confidence interval for the ε value calculated for the experiment using DHB-CF/MEL without methanogens was larger (±2.7‰), the ε value was still within error of those for the DHB-CF/MEL with methanogens. Hence, an overall ε value is calculated for all four experiments of −27.5 ± 0.9 ‰ (Table 1).

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

		ε value		
compound	replicate no.	(‰)	r^2	95% CI
DHB-CF/MEL with methanogens	1	−26.8	0.99	1.2
	2	−27.6	0.99	1.6
	3	−28.0	0.99	1.8
	all	−27.6	0.99	0.9
DHB-CF/MEL without methanogens	1	−28.5	0.99	2.7
	all 4 replicates	−27.5	0.97	0.9

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

DISCUSSION

To facilitate comparison of measured enrichment factors (ε) between different compounds, it is helpful to express the magnitude of fractionation in terms of apparent kinetic isotope effects (AKIE). AKIE is the position-specific kinetic isotope effect (KIE) accounting for differences in the number of carbon atoms in the molecule (n), the number of carbon atoms in reacting positions (x) and the number of carbon atoms in chemically equivalent positions (z) as described by[34]

$$\frac{1}{\mathrm{AKIE_C}} = \frac{z \cdot n \cdot \varepsilon_{\mathrm{bulk}}}{x \cdot 1000} + 1 \quad (1)$$

For compounds with only one carbon atom such as CF $n = x = z = 1$, the measured ε value (ε = −27.5 ± 0.9‰) can be converted directly to an AKIE value of 1.028 (Table 2). Such AKIE can be compared to the maximum theoretical intrinsic KIE value, which in the case of C−Cl bond cleavage is typically estimated at 1.03.[34] The measured AKIE during biodegradation of CF by DHB-CF/MEL (1.028) agrees with the intrinsic KIE within uncertainty. While no experiments determining KIE for abiotic degradation of CF have been published to date, several

Table 2. Measured ε Values and Calculated AKIE Values of CF, CT, 1,1,1-TCA for Biotic and Abiotic Degradation

compound	condition	ε value	AKIE
CF	biotic; this study	−27.5	1.028 ± 0.002
CT[a]	abiotic	−26.1 to −26.5	1.027 ± 0.002
1,1,1-TCA[b]	biotic	−1.8 to −1.5	1.0036 ± 0.0006
1,1,1-TCA[c]	abiotic	−15.8 to −13.6	1.027 ± 0.002

[a]Values obtained for experiments of abiotic reductive cleavage of CT by Fe (II)/ goethite and Fe(II) porphyrin.[28] [b]Values obtained by biotic reductive cleavage of 1,1,1-TCA by a *Dehalobacter*-containing culture.[6] [c]Values obtained for experiments of abiotic reductive cleavage of 1,1,1-TCA by Cr(II), Fe^0, and Cu and Fe mixtures.[10] AKIE for CF are calculated from ε values as described in text.

studies have reported carbon isotope effects during abiotic degradation of the related compound, CT. In a series of different experiments, even though CT was dechlorinated by a variety of iron hydroxide minerals (goethite, hematite, leipidocrocite, magnetite, and siderite), the observed ε values for different iron minerals were all similar at −33‰ to −27‰.[34] This too corresponds to a KIE consistent with theoretical predictions of the intrinsic KIE associated with cleavage of a single C−Cl bond. While isotopic fractionation effects during abiotic degradation of CF have yet to be carried out, it is likely they would be very similar to those published for CT.

As noted earlier, carbon isotope fractionation during biodegradation of 1,1,1-TCA (n = 2; x = z=1) by the same culture (DHB-CF/MEL) shows significantly different results, specifically a lack of agreement between observed AKIE and theoretical intrinsic KIE values. While abiotic degradation of 1,1,1-TCA, like abiotic degradation of CT and biodegradation of CF, has an AKIE that agrees with the intrinsic KIE of 1.03 (Table 2), carbon isotope fractionation of 1,1,1-TCA by DHB-CF/MEL is an order of magnitude smaller (AKIE of 1.0036 ± 0.002). Sherwood Lollar et al.[6] showed that this small AKIE can be attributed to masking of the intrinsic KIE due to the increased enzyme efficiency of the *Dehalobacter* enzyme catalyzing the 1,1,1-TCA dechlorination. A model by Northrup et al.[38] describes how enzyme kinetics may affect isotopic fractionation in biochemical reactions:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P \quad (2)$$

where E represents the enzyme, S represents the substrate, ES represents the enzyme−substrate complex, and P represents the products. First order rate constants for the formation and dissociation of the enzyme−substrate complex are k_1 and k_{-1}, respectively, and k_2 is the rate constant for the irreversible chemical transformation of the substrate. The relationship between apparent kinetic isotope effect (AKIE), the intrinsic isotope (KIE) and individual rate constants during an enzymatic reaction is given by eq 3:

$$\text{AKIE} = (\text{KIE} + C)/(1 + C) \quad (3)$$

where the commitment factor ($C = k_2/k_{-1}$) describes the tendency of the ES-complex to proceed forward to products rather than back to free enzyme and substrate. In the case of a highly efficient enzyme as observed during 1,1,1-TCA degradation by the DHB-CF/MEL culture, the high rate of k_2 compared to the reverse reaction k_{-1} results in a large C factor and the AKIE for the experiment is significantly masked compared to the intrinsic KIE associated with C−Cl bond cleavage.[38]

Intriguingly, no similar masking effect was observed during biodegradation of CF. Various possibilities may explain this finding. While the same *Dehalobacter* reductive dehalogenase catalyzes both CF and 1,1,1-TCA dechlorination by the DHB-CF/MEL culture,[12] the protein structure of any reductive dehalogenase has yet to be determined. Because of the lack of a structure and attendant understanding of mechanistic details for reductive dehalogenase reactions, it is not possible at this time to measure differences in substrate binding orientation and affinity for CF versus 1,1,1-TCA that may explain the observed differences in fractionation. However, the two compounds differ significantly in physical and chemical properties that are likely to influence binding and reactivity. 1,1,1-TCA has a larger Henry's Law constant (H = 0.7 dimensionless) and octanol−water partition coefficient (log K_{ow} = 2.48) compared to CF (H = 0.15 dimensionless and log K_{ow} = 1.92), reflecting the lower aqueous solubility and higher hydrophobicity of 1,1,1-TCA.[39] Since the enzyme−substrate binding pocket is likely to be quite hydrophobic to accommodate the three chlorine substituents on the substrates, 1,1,1-TCA may bind more tightly than CF and if carried through in the rate-determining transition state could account for differences in observed fractionation.[40] While the specific reason for the different expression of KIE for CF and 1,1,1-TCA cannot be resolved at this time, the finding is significant. We show that for similar reactions all involving a single C−Cl cleavage, that subtle differences in the details of the enzyme−substrate interactions can have profound effects on the observed fractionation compared to abiotic and theoretical KIE. This was shown both in systems where the same enzyme acts upon two different substrates (1,1,1-TCA and CF) - this study); and where two distinct yet similar enzymes act on two similar substrates (1,1,1-TCA and 1,1-DCA).[6,33] Further studies will attempt to explore these hypotheses by purifying the enzymes responsible for CF, 1,1,1-TCA and 1,1-DCA degradation.

Environmental Significance. The results of this study demonstrate a large fractionation associated with biodegradation of CF that is consistent with the theoretically predicted intrinsic KIE for a single C−Cl bond cleavage (1.03). Such a fractionation is large (ε = −27.5‰) and comparable to those established for cDCE and VC and hence provides the foundation for using CSIA as an approach to identify and monitor biodegradation as has been done for the chlorinated ethenes.[2]

Recent studies have demonstrated that industrial sources are not the only contributors of CF to the environment[41] although the role of natural abiotic versus biotic processes in the soil zone is still debated.[42,43] Through compound specific isotope experiments, Breider et al.[44] demonstrated that chlorination of naturally occurring organic matter (particularly trichloroacetyl-containing compounds) can produce an enriched δ^{13}C value for soil zone CF. Hunkeler et al.[45] suggested that this significantly more ^{13}C-enriched value (on average −25 ‰) may permit natural soil zone CF to be distinguished from industrial CF (published δ^{13}C values −65‰ to −45‰; [2]), that are present due to anthropogenic activities. Our experiments demonstrate however that δ^{13}C values as enriched as −25 ‰ can be produced from biodegradation of industrial CF, underscoring the need to evaluate source signatures at contaminated field sites with care. While CSIA can be a valuable approach for source fingerprinting of hydrocarbon contaminants,[45,46] the δ^{13}C data must be interpreted within the detailed hydrogeological and the geological context of the site.

AUTHOR INFORMATION

Corresponding Author

*E-mail: bslollar@chem.utoronto.ca.

Notes

The authors declare no competing financial interest.

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