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Carbon and Chlorine Isotope Fractionation during Aerobic Oxidation and Reductive Dechlorination of Vinyl Chloride and *cis*-1,2-Dichloroethene

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The study investigated carbon and chlorine isotope fractionation during aerobic oxidation and reductive dechlorination of vinyl chloride (VC) and *cis*-1,2-dichloroethene (cDCE). The experimental data followed a Rayleigh trend. For aerobic oxidation, the average carbon isotope enrichment factors were −7.2‰ and −8.5‰ for VC and cDCE, respectively, while average chlorine isotope enrichment factors were only −0.3‰ for both compounds. These values are consistent with an initial transformation by epoxidation for which a significant primary carbon isotope effect and only a small secondary chlorine isotope effect is expected. For reductive dechlorination, larger carbon isotope enrichment factors of −25.2‰ for VC and −18.5‰ for cDCE were observed consistent with previous studies. Although the average chlorine isotope enrichment factors were larger than those of aerobic oxidation (−1.8‰ for VC, −1.5‰ for cDCE), they were not as large as typically expected for a primary chlorine isotope effect suggesting that no cleavage of C−Cl bonds takes place during the initial rate-limiting step. The ratio of isotope enrichment factors for chlorine and carbon were substantially different for the two reaction mechanisms suggesting that the reaction mechanisms can be differentiated at the field scale using a dual isotope approach.

Introduction

The use of stable isotope analysis has become an important tool to assess degradation of organic compounds at contaminated sites (*1*). The approach relies on isotope fractionation caused by a more rapid transformation of molecules with light isotopes compared to those with one or several heavy isotopes. As a result, the remaining contaminant pool becomes progressively enriched with the heavy isotopes. The decreasing contaminant concentration and increasing isotope ratios can be mathematically related by the Rayleigh equation with the isotope enrichment factor ϵ as the key parameter (*2*). Since the magnitude of the isotope enrichment factor depends on the initial transformation step, the reaction mechanism needs to be known for a quantitative interpretation of field isotope data. Although different mechanisms are generally associated with a different ϵ, it is usually not possible to identify the reaction mechanism based on isotope data for a single element only (*1*). Contaminant concentrations are usually also affected by physical processes in addition to transformation, so the calculation of supposed field-based isotope enrichment factors is not a reliable practice (*3, 4*). In addition, if a compound is degraded by different pathways simultaneously at a site, the observed isotope fractionation reflects the mixed effect of individual isotope fractionations caused by these pathways. Since different reaction mechanisms frequently involve different bonds during the initial step, the measurement of multiple isotopes has been proposed as a method to identify reaction mechanisms of organic contaminants and/or quantify the relative contribution of two pathways (*5*−*11*). This approach is well established for evaluating the origin and fate of inorganic compounds (NO_3^-, SO_4^{2-}) and is usually denoted as the dual isotope approach (*2*). For organic compounds, the term two-dimensional isotope approach has been used as well. The premise of the dual isotope approach is that by measuring the isotope ratios of two elements of a compound simultaneously (such as $^{13}C/^{12}C$ and $^{2}H/^{1}H$ or $^{13}C/^{12}C$ and $^{37}Cl/^{35}Cl$), a correlation between isotope fractionation of two elements is observed that is characteristic of the reaction mechanism. Unlike a field-based calculation of ϵ-values based on the Rayleigh fractionation model (*1*), such a correlation is independent of contaminant concentrations and therefore can assist in elucidating the responsible reaction mechanism. This dual isotope approach was successfully employed to identify the mechanism of methyl *tert*-butyl ether transformation at the field scale (*10, 12*). Furthermore, laboratory studies indicate that it may also be useful to identify the mechanism of benzene degradation based on combined carbon and hydrogen isotope analysis (*13*). However, it has to be taken into account that even for given redox conditions, compounds may be degraded by a different mechanism leading to different ratios of isotope fractionation of two elements (*13, 14*). Once the reaction mechanism at a contaminated site is identified, typical Rayleigh-type interpretations of field isotope data can be carried out. The quantification has to take into account potential variations in isotope fractionation for a given pathway.

The present study aims at evaluating the potential of the dual isotope approach for analyzing the fate of vinyl chloride (VC) and *cis*-dichloroethene (cDCE) as they are degraded or transformed by a number of different pathways. VC and cDCE can be reductively dechlorinated by microorganisms under strongly reducing conditions or oxidized under aerobic and anaerobic conditions (*15*). Furthermore, abiotic reduction of cDCE and VC by iron-bearing minerals has been reported (*16*). This study compares the effect of reductive dechlorination and aerobic oxidation of cDCE and VC on carbon and chlorine isotope fractionation. Significant carbon isotope fractionation was previously reported for aerobic oxidation of VC (−3.2 to −8.2‰) (*17, 18*), for reductive dechlorination of VC (−22.4 to −31.1‰) (*19*−*21*), and for reductive dechlorination of cDCE (−14.1 to −20.4‰) (*19, 21*) while no data are available so far for aerobic cDCE degradation. Aerobic

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oxidation of VC involves the formation of an epoxide at the carbon double bond as an initial rate-limiting step (*22*−*26*). Since the C−Cl bonds remain intact during the initial step, only a secondary chlorine isotope effect is expected, caused by the presence of a heavy isotope in a position adjacent to the reactive center. For reductive dechlorination of cDCE and VC, the precise reaction mechanism is not known yet. However, studies with corrinoids as reductants provide clues about potential reaction mechanisms. The initial step may involve a dissociative one-electron transfer, which would likely lead to significant chlorine isotope fractionation due to the cleavage of a C−Cl bond. Alternatively, an ethylcobalamin may be formed by simultaneous nucleophilic addition of cob(I)alamin to one of the carbons and protonation of the other, followed by a rapid reductive dissociation into the dechlorinated ethene and free chlorine (*27*−*29*). If the former step is rate-limiting, a small chlorine isotope effect is expected because no C−Cl bond will have been broken while when the latter is rate-limiting, a substantial chlorine isotope effect is expected, similar to the first mechanism.

In this study, carbon and chlorine isotope fractionation was investigated using batch systems with pure (aerobic oxidadation) and mixed (reductive dechlorination) cultures. Isotope enrichment factors for carbon (ϵ_C) and chlorine (ϵ_{Cl}) for each pathway were measured to evaluate if ratios between chlorine and carbon isotope fractionation varied for different reaction mechanisms. To gain additional insight into the factors that control the magnitude of isotope fractionation, the apparent kinetic isotope effect (AKIE) was calculated based on the scheme proposed by Elsner et al. (*1*) and compared to expected values for different mechanisms.

Materials and Methods

Aerobic Oxidation Experiment. Aerobic VC oxidation experiments used the *Nocardioides* strain JS614 (*30*), and aerobic cDCE experiments used the β-Proteobacterium strain JS666 (*31*). Both strains were kindly provided by Dr. Spain's laboratory (Georgia Institute of Technology, Atlanta, GA). The culture medium was prepared as described elsewhere (*30, 31*). The stock cultures were kept in 250 mL glass bottles with 100 mL headspace and capped with Mininert-Valves (Vici Precision Sampling, Baton Rouge, LA). They were placed continuously on a rotary shaker. The initial VC (>99.5% purity, Fluka, Switzerland) and cDCE (>99.5% purity, Fluka, Switzerland) concentrations were 0.5 mM in the headspace. The headspace substrate concentrations were regularly measured with a gas chromatograph (GC) equipped with a flame ionization detector (model 4130, Carlo Erba Strumentazione, Italy). Calibration was performed using external standards. For the experiment, 15-mL portions of the stock culture were added to 60-mL serum bottles containing 5 mL of fresh medium, capped with a Viton stopper, and amended with either VC (0.5 mM in the liquid phase) or cDCE (2 mM). In total, 10 replicas of 60-mL bottles containing 20 mL of the culture solution were prepared for both the VC and the cDCE series and placed on a rotary shaker to enhance air−water phase partitioning. As a negative control, one of the 10 bottles from both the VC and the cDCE series was inactivated with sodium azide to a final w/w concentration of 1% before adding the substrate. After two hours of shaking, initial substrate concentrations were measured. Headspace substrate concentrations were monitored throughout the experiment and aqueous phase concentration was calculated using Henry's law and Henry coefficients (*32*). During the course of the experiment, each time a multiple of a half-log decrease in normalized concentration was observed, one of the bottles was inactivated by adding sodium azide and kept at 4 °C for isotope analyses. All experiments were carried out at 23 ± 1 °C.

Anaerobic Reductive Dechlorination Experiments. The reductive dechlorination experiments for VC and cDCE were carried out at the SiREM Laboratory located in Guelph, Ontario, Canada. KB-1, a commercially available natural dechlorinating culture containing *Dehalococcoides* (Dhc) organisms was used for both experiments. For each substrate tested, three sets of vials sealed with Mininert screw caps (VICI Precision Sampling, Inc., Baton Rouge, LA) were filled with anaerobic mineral salts medium for the duplicate experiments. Sterile control vials were prepared by amending the vials with mercuric chloride and sodium azide to inhibit microbial activity and were used to quantify potential abiotic and experimental losses from the vials. The starting VC and cDCE concentrations in the liquid phase were adjusted to 1.0 mM for both VC and cDCE. Vials were kept at 22 °C and sampled over a 7-day period for chlorinated ethenes and ethene concentrations. Aqueous chlorinated ethene and ethene concentrations in the microcosms were measured using a Hewlett-Packard (5890 series II Plus) gas chromatograph equipped with an auto sampler (Hewlett-Packard 7684). Calibration was performed using external standards purchased as standard solutions (Sigma, St. Louis, MO).

Isotope Ratio Analysis. Carbon isotope ratios of VC and cDCE were analyzed by GC coupled to an isotope-ratio mass spectrometer (IRMS) via a combustion interface (Thermo Finnigan, Germany). The system was equipped with a purge-and-trap concentrator (Velocity XPT, Tekmar-Dohrmann, USA) connected to the GC via a cryogenic trap. Required concentrations for VC and cDCE carbon isotope analysis were approximately 10 and 5 μg/L, respectively. Samples were diluted to VC and cDCE concentrations of approximately 30 and 15 μg/L, respectively, for the carbon isotope analysis. Chlorine isotope ratios of VC and cDCE were analyzed by a continuous flow-isotope ratio mass spectrometer (Iso Prime, Micromass, currently GV Instruments) equipped with a gas chromatograph (Agilent 6890) and a CombiPAL SPME autosampler (CTC analytical, Switzerland) as described elsewhere in detail (*33, 34*). All isotope ratios are reported using the delta notation as $\delta = (R/R_{std} - 1) \times 1000‰$ where R and R_{std} are the isotope ratio of the sample and the standard, respectively. All $\delta^{13}C$ values are reported relative to Vienna PeeDee Belemnite ($\delta^{13}C_{VPDB}$) and $\delta^{37}Cl$ values are reported relative to Standard Mean Ocean Chloride ($\delta^{37}Cl_{SMOC}$). Dilution of the samples to similar concentrations led to high precision measurements with a standard deviation of 0.3‰ for carbon isotopes and 0.1‰ for chlorine isotopes.

Calculation Method

Quantification of Isotope Fractionation. Carbon isotope enrichment factors were quantified using the Rayleigh equation, which corresponds to

$$\ln \frac{(1000 + \delta^{13}C)}{(1000 + \delta^{13}C_0)} = \frac{\varepsilon}{1000} \ln \frac{C}{C_0} \quad (1)$$

where $\delta^{13}C$ is the measured isotope ratio at time t, $\delta^{13}C_0$ is the initial isotope ratio, C is the substrate concentration at time t, C_0 is the initial concentration, and ε the isotope enrichment factors. The isotope enrichment factor is related to the isotope fractionation factor α by $\varepsilon = (\alpha - 1) \cdot 1000$. Recently, it was demonstrated that an analogous approach can also be used to evaluate chlorine isotope data although the heavy chlorine isotope is present at a much higher abundance than for carbon (*35*). Equation 1 can be approximated by

$$\delta^{13}C = \delta^{13}C_0 + \varepsilon \cdot \ln \frac{C}{C_0} \quad (2)$$

The division of eq 2 by an analogous expression with respect to chlorine isotope yields

$$\frac{\Delta\delta^{37}\mathrm{Cl}}{\Delta\delta^{13}\mathrm{C}} = \frac{\varepsilon_{\mathrm{Cl}}}{\varepsilon_{\mathrm{C}}} \quad (3)$$

where $\Delta\delta^{37}$Cl and $\Delta\delta^{13}$C are the differences in carbon and chlorine isotope ratios, respectively, between time t and zero. The equation demonstrates that the slope of a δ^{37}Cl to δ^{13}C plot should correspond to the ratio between the isotope enrichment factors for the two isotopes.

Calculation of Apparent Kinetic Isotope Effects. A kinetic isotope effect (KIE) is caused by differences in reaction rate constants for molecules containing a heavy and light isotope, respectively, in the reacting bond and is defined as follows:

$$\mathrm{KIE} = \left(\frac{{}^{L}k}{{}^{H}k}\right) \quad (4)$$

where ${}^{L}k$ and ${}^{H}k$ are the reaction rate constants of molecules with a light and a heavy isotope, respectively. During compound-specific isotope analysis, the average isotope ratio of the molecule is determined rather than the isotope ratios at a specific position and bulk isotope fractionation (α_{bulk}) factors are obtained when evaluating the data according to the Rayleigh equation. Due to the presence of nonreacting positions in most molecules, the bulk isotope fractionation factors are smaller than expected based on eqn 4 and depend on the molecule size. To be able to compare the magnitude of isotope effect for compounds regardless of their molecular size and structure, a scheme to estimate position-specific isotope effects from bulk isotope data has been proposed (*1*). The obtained position-specific isotope effects are denoted

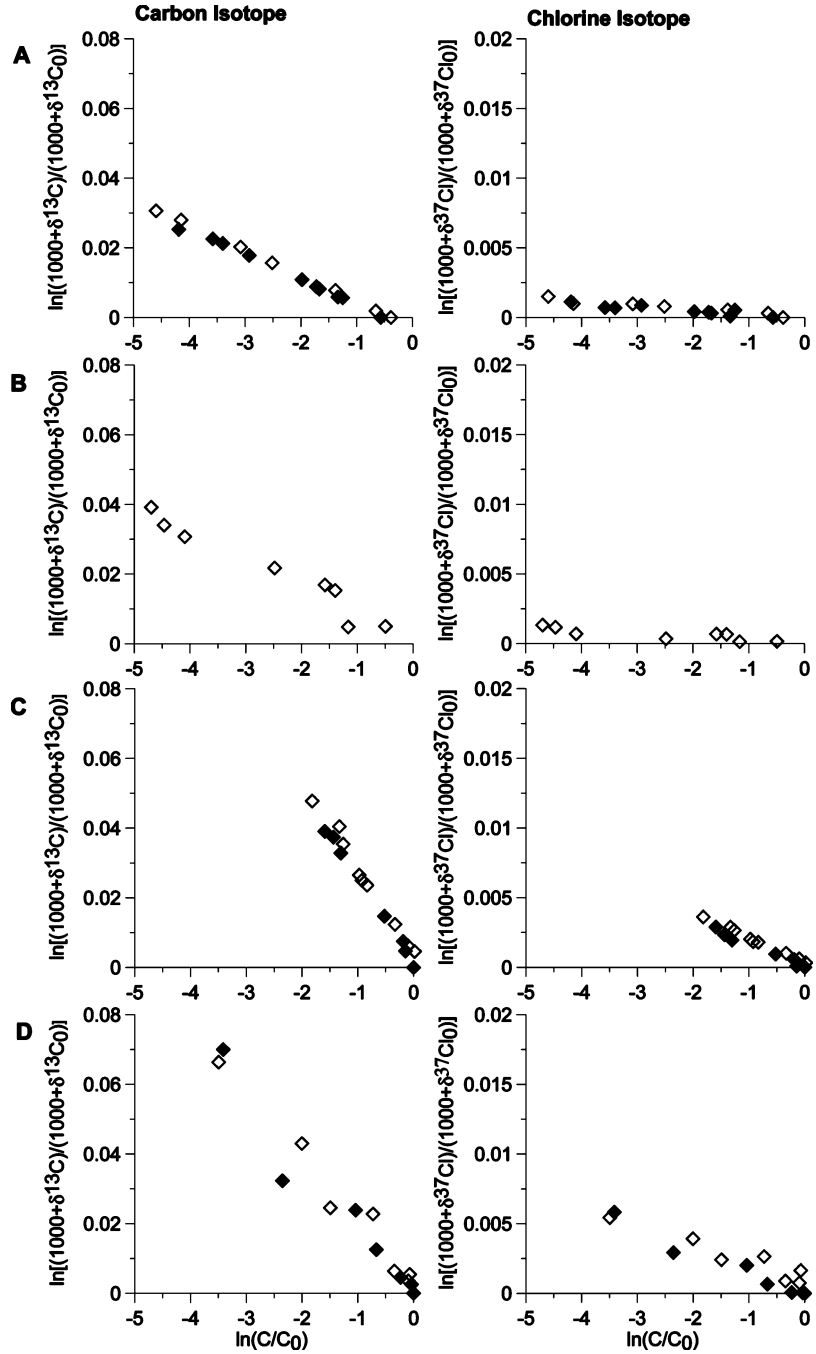

FIGURE 1. Rayleigh plots for four experiments: (A) aerobic VC oxidation, (B) aerobic cDCE oxidation, (C) VC reductive dechlorination, and (D) cDCE reductive dechlorination. The open and closed symbols correspond to two replicates. Note that the *y*-scale is different for carbon and chlorine isotopes.

TABLE 1. Bulk Isotope Enrichment Factors Measured in This Study and Previously Reported Values (±Values Correspond to the Standard Deviation for the Isotope Enrichment Factors Obtained by Linear Regression)

		observed	reported	
		ϵ_{bulk} [‰]	ϵ_{bulk} [‰]	ref
VC oxidation	C	−7.2 ± 0.16, −7.3 ± 0.07	−3.2 to −8.2	(*17, 18*)
	Cl	−0.3 ± 0.04, −0.3 ± 0.04		
cDCE oxidation	C	−8.5 ± 0.10		
	Cl	−0.3 ± 0.07		
VC reductive dechlorination	C	−25.0 ± 0.72, −25.4 ± 1.10	−22.4 to −31.1	(*19*−*21*)
	Cl	−1.7 ± 0.12, −1.9 ± 0.08		
cDCE reductive dechlorination	C	−18.5 ± 1.80, −18.5 ± 1.30	−14.1 to −20.4	(*19, 21*)
	Cl	−1.6 ± 0.14, −1.4 ± 0.20		
DCM hydrolytic dehalogenation	C		−42.4	(*8*)
	Cl		−3.8	(*8*)

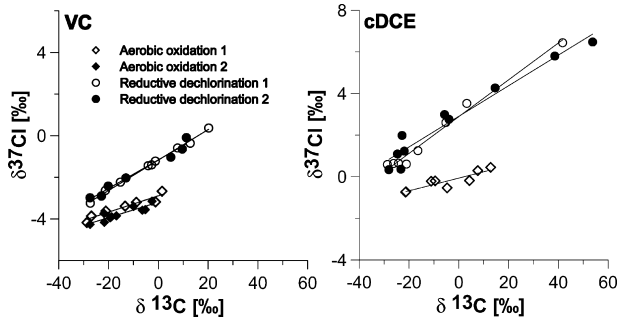

FIGURE 2. Comparison of $\delta^{13}C$ vs $\delta^{37}Cl$ plots for VC and cDCE experiments.

TABLE 2. Comparison of Slope of $\delta^{13}C$ vs. $\delta^{37}Cl$ Graph with Ratio of Isotope Enrichment Factors for Chlorine and Carbon (±Values Correspond to the Standard Deviation for the Slope Obtained by Linear Regression)

	slope $\delta^{13}C$ vs $\delta^{37}Cl$	ϵ_{Cl}/ϵ_C
VC aerobic oxidation	0.039 ± 0.0055, 0.037 ± 0.0063	0.042
cDCE aerobic oxidation	0.031 ± 0.0061	0.035
VC reductive dechlorination	0.073 ± 0.0015, 0.069 ± 0.0050	0.071
cDCE reductive dechlorination	0.088 ± 0.0044, 0.073 ± 0.0058	0.081
DCM oxidation		0.09

as apparent kinetic isotope effect (AKIE) because they can be smaller than the actual KIE for reasons given below.

According to this procedure, the presence of nonreacting positions within a molecule can be corrected using (*1*, *36*)

$$\alpha_{rp} - 1 \approx \frac{n}{x} \cdot (\alpha_{bulk} - 1) \quad (5)$$

where α_{rp} is the reactive-position specific fractionation factor, n is the number of the atoms of the element considered in a molecule, x is the number of atoms of the considered element located at the reacting site, and α_{bulk} is the measured isotope fractionation factor. The presence of multiple identical reacting positions is taken into account using (*1*)

$$\text{AKIE} = \frac{1}{1 + z \cdot \varepsilon_{rp}/1000} \quad (6)$$

where z is the number of atoms in identical reacting positions that are in intramolecular competition, ϵ_{rp} is the reacting-position-specific ϵ. AKIE values can still be lower than its KIE values if (1) the bond is not completely broken in the transition state, (2) the transport of a substrate across the cell membrane to an enzyme is the rate-determining step as shown previously for the case of PCE reductive dechlorination (*37*), and/or the catalytic step is fast compared to the preceding binding steps (*38*). The relative velocity of catalytic to binding steps is often expressed as the *commitment to catalysis* which corresponds to the ratio between the rate of the catalytic step to the rate of substrate-enzyme dissociation (*38*). The larger the commitment to catalysis, the smaller the value for AKIE

$$\text{AKIE} = \frac{\text{KIE} + C}{1 + C} \quad (7)$$

where C is the commitment to catalysis.

Comparison of AKIE Values for Different Elements. The possible difference between the AKIE and KIE values (eq 7) complicates the comparison of AKIE with theoretical KIE values for a reaction. However similar to the effect of dilution in open systems, which cancels out when calculating ratios of isotope enrichment factors for two elements (see above), the commitment to catalysis cancels in approximation (1) if ratios of AKIE-1 are compared

$$\frac{\text{AKIE}_{Cl} - 1}{\text{AKIE}_{C} - 1} = \frac{(\text{KIE}_{Cl} + C - 1 - C)/(1 + C)}{(1 + C)/(\text{KIE}_{C} + C - 1 - C)} = \frac{\text{KIE}_{Cl} - 1}{\text{KIE}_{C} - 1} \quad (8)$$

According to this equation AKIE-1 ratios calculated based on experimental data using eq 6 should be similar to KIE-1 ratios calculated based on data from abiotic reference experiments or theoretical considerations even if commitment to catalysis occurs. Furthermore, AKIE-1 ratios for different compounds undergoing the same reaction should be comparable.

Results and Discussion

Bulk Isotope Fractionation. In Figure 1, the carbon and chlorine isotope data were plotted according to the Rayleigh equation (eq 1). Negative controls did not demonstrate significant concentration decreases nor changes in their carbon and chlorine isotope ratios (data not shown). Carbon isotope fractionation was much larger than chlorine isotope fractionation for all four experiments. In addition, carbon and chlorine isotope fractionation was larger for reductive dechlorination than for aerobic oxidation. The bulk enrichment factors (ϵ_{bulk}) are summarized in Table 1. Statistical tests revealed no significant difference between ϵ_{bulk} values for duplicate experiments at the 95%

TABLE 3. AKIE Values for Carbon and Chlorine Isotopes and Their AKIE-1 Ratios Calculated for Different Reaction Mechanisms[a]

		ϵ_{bulk}	*n*	*x*	*z*	AKIE	$(AKIE_{Cl} - 1)/(AKIE_C - 1)$	*n*	*x*	*z*	AKIE	$(AKIE_{Cl} - 1)/(AKIE_C - 1)$
						epoxidation						
VC oxidation	C	−7.2	2	2	1	1.0073	0.04					
	Cl	−0.3	1	1	1	1.0003						
						epoxidation [b]						
cDCE oxidation	C	−8.5	2	2	1	1.0085	0.04					
	Cl	−0.3	2	2	1	1.0003						
						mechanism I/mechanism IIb					mechanism IIa	
VC reductive dechlorination	C	−25.2	2	1	1	1.0515	0.03	2	2	1	1.0256	0.07
	Cl	−1.8	1	1	1	1.0017		1	1	1	1.0017	
						mechanism I/mechanism IIb					mechanism IIa	
cDCE reductive dechlorination	C	−18.5	2	2	2	1.0384	0.08	2	2	1	1.0188	0.08
	Cl	−1.5	2	2	2	1.0030		2	2	1	1.0015	
						nucleophilic substitution S_N2						
DCM dechlorination	C	−42.4	1	1	1	1.0443	0.17					
	Cl	−3.8	2	2	2	1.0077						

[a] For explanations of Mechanisms I, IIa und IIb see text. *n*: number of atoms of element present; *x*: number of reacting sites; *z*: number of reacting sites in intramolecular competition. [b] Exact reaction mechanism has not yet been identified.

confidence level. The carbon isotope enrichment factors for aerobic oxidation of VC, reductive dechlorination of VC, and reductive dechlorination of cDCE were within the range reported in previous studies (Table 1). For aerobic oxidation of cDCE, carbon and chlorine isotope enrichment factors were similar to those for VC suggesting that degradation may proceed by the same mechanism although the mechanism for aerobic cDCE oxidation is not yet known. Chlorine isotope fractionation was particularly small for aerobic oxidation of VC and cDCE with the same $\epsilon_{bulk,Cl}$ value of −0.3‰. The VC oxidation reaction is initiated by the formation of an epoxide as the rate-determining step which does not involve a cleavage of C−Cl bond (*23, 31*); hence, only a small chlorine isotope effect is expected. Reductive dechlorination of VC and cDCE resulted in a larger $\epsilon_{bulk,Cl}$ values (on average −1.8 and −1.5‰ for VC and cDCE, respectively). The carbon and chlorine isotope enrichment factors found for reductive dechlorination were smaller than those previously reported for aerobic biodegradation of dichloromethane (*8*), which involves an S_N2 nucleophilic substitution of a chlorine atom (Table 1). They are also smaller than for reductive beta-elimination of chlorinated ethanes (*11*).

Correlation between Carbon and Chlorine Isotope Fractionation. Figure 2 illustrates the relationship between the carbon and chlorine isotope evolution. For all experiments, a linear relation between $\delta^{13}C$ and $\delta^{37}Cl$ values was observed. As expected based on eq 3, the slopes of the $\delta^{13}C-\delta^{37}Cl$ plots corresponded well to the ϵ_{Cl}/ϵ_C ratios (Table 2). The $\delta^{13}C-\delta^{37}Cl$ slopes of aerobic oxidation of VC and cDCE were approximately half as large as those of VC and cDCE reductive dechlorination and the ϵ_{Cl}/ϵ_C ratio of DCM dehalogenation. Statistical tests confirmed that there was a significant difference in the slope between aerobic oxidation and reductive dechlorination for both VC and cDCE at the 95% confidence level. The significant difference in slope suggests that the two processes can be distinguished using a dual isotope approach. While dual isotope plots can potentially help to distinguish between two reaction mechanisms, they do not provide insight into the respective reaction mechanisms before corrections for nonreacting positions are made.

Apparent Kinetic Isotope Effects. To evaluate more systematically how the magnitude of isotope fractionation is related to the reaction mechanism, AKIE values and the AKIE-1 ratios for different elements were calculated (Table 3). The calculations were based on average ϵ_{bulk} values for duplicate experiments. Depending on the reaction scenario considered a different AKIE will be obtained because the number of reacting and nonreacting positions varies.

For aerobic oxidation of VC and cDCE, the calculated $AKIE_C$ values for epoxidation were similar to values measured in an abiotic reference experiment (1.011 (*39*)). No abiotic reference data for Cl isotopes and epoxidation are available. However, the small AKIE is consistent with a secondary chlorine isotope effect expected for epoxidation. The obtained $AKIE_{Cl} - 1$ is 43 times smaller than the expected $AKIE_{Cl} - 1$ for a complete breaking of a typical C−Cl bond according to the Streitwieser semiclassical limit (*40*).

Since the exact reaction mechanism is not yet known for reductive dechlorination of VC and cDCE, two scenarios were considered. Mechanism I consists of a dissociative one-electron transfer mechanism (mainly proposed for PCE and TCE reductive dechlorination (*41*)) which involves only one carbon and chlorine during C−Cl bond cleavage to form a radical. Thus a primary KIE is expected for both elements. Assuming complete breaking of the C−Cl bond in the transition state, a KIE_C of 1.057, a KIE_{Cl} of 1.013, and $(KIE_{Cl} - 1)/(KIE_C - 1)$ ratio of 0.23 would be expected according to the Streitwieser semiclassical limit. If only partial cleavage occurs or commitment to catalysis is significant, the actual KIE values would be somewhat smaller while the AKIE − 1 ratio is expected to remain around 0.23. While the calculated $AKIE_C$ values for reductive dechlorination of VC and cDCE were close to the expected value (Mechanism I, Table 3), the $AKIE_{Cl}$ values were substantially smaller than expected. Furthermore, the AKIE − 1 ratios were substantially smaller compared to the value for C−Cl bond cleavage suggesting that this reaction mechanism is unlikely. It is interesting to note that the AKIE − 1 ratio for hydrolytic dehalogenation of DCM, which is known to involve cleavage of a C−Cl bond, was close to the theoretical ratio of 0.23.

Mechanism II corresponds to a nucleophilic attack, which acts on one of the carbons accompanied by a simultaneous protonation of the other followed by reductive dissociation into the dechlorinated ethene and a free chlorine (*28, 29*). If the first step is rate-limiting (Mechanism IIa), a primary isotope effect with respect to carbon and a secondary with respect to chlorine is expected and the average isotope effect for the two positions would be

observed. If the second step is rate-limiting (Mechanism IIb), a primary carbon and chlorine isotope effect similar to the first mechanism should be observed possibly enhanced by an equilibrium isotope effect associated with the first step. Again, similarly as for Mechanism I, for Mechanism IIb, the $AKIE_{Cl}$ values (1.0017 for VC, 1.003 for cDCE) were significantly smaller than expected for a C−Cl cleavage (1.013) indicating that this mechanism is unlikely. For mechanisms IIa, $AKIE_{Cl}$ values were 8 (VC) or 9 (cDCE) times smaller than the reference value for C−Cl cleavage given above, suggesting that chlorine isotope fractionation may be due to a secondary isotope effect compatible with Mechanism IIa. All calculated $AKIE_{Cl}$ values were smaller than estimated KIE (1.0055 to 1.0074 (*42*)) for reductive dechlorination of trichloroethene (TCE) and tetrachloroethene (PCE) suggesting that cDCE and VC are degraded by a different mechanism than TCE and PCE. A more thorough and quantitative assessment of the compatibility of this reaction scenario with the observed isotope fractionation would require the simulation of the transition state structure using computational methods, which is beyond the scope of this paper.

Implications for Assessment of Processes at Contaminated Sites. The study demonstrates that the ratio between carbon and chlorine isotope fractionation is significantly different for reductive dechlorination and aerobic oxidation opening the possibility to distinguish between the two processes using a dual isotope approach. The dual isotope approach could be particularly interesting for evaluating the fate of VC and cDCE at plume fringes where oxygen may become available again. Since contaminant concentrations are strongly affected by dispersion at plume fringes and oxygen may be rapidly consumed it may be difficult to demonstrate the processes based on concentration data. The dual isotope approach is potentially also applicable to demonstrate anaerobic oxidation of VC and cDCE. However, no pure or enrichment cultures are currently available and the reaction mechanism is unknown, making it impossible to characterize the expected isotope trends at this time.

Acknowledgments

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