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Chlorine Isotope Fractionation during Reductive Dechlorination of Chlorinated Ethenes by Anaerobic Bacteria

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Chlorine isotope fractionation during reductive dechlorination of trichloroethene (TCE) and tetrachloroethene (PCE) to *cis*-1,2-dichloroethene (cDCE) by anaerobic bacteria was investigated. The changes in the $^{37}Cl/^{35}Cl$ ratio observed during the one-step reaction (TCE to cDCE) can be explained by the regioselective elimination of chlorine accompanied by the Rayleigh fractionation. The fractionation factors (α) of the TCE dechlorination by three kinds of anaerobic cultures were approximately 0.994−0.995 at 30 °C. The enrichment of ^{37}Cl in the organic chlorine during the two-step reaction (PCE to cDCE) can be explained by the random elimination of one chlorine atom in the PCE molecule followed by the regioselective elimination of one chlorine atom in the TCE molecule. The fractionation factors for the first step of the PCE dechlorination with three kinds of anaerobic cultures were estimated to be 0.987−0.991 at 30 °C using a mathematical model. Isotope fractionation during the first step would be the primary factor for the chlorine isotope fractionation during the PCE dechorination to cDCE. The developed models can be utilized to evaluate the fractionation factors of regioselective and multistep reactions.

Introduction

Chlorinated aliphatic hydrocarbons (CAHs) have been widely utilized as solvents and degreasing agents for many years and have caused the wide and long-lasting pollution of surface soils, unsaturated zones, and aquifers. Although CAHs are degraded by abiotic or biotic reactions (*1*), the reaction rates are usually low in natural systems.

As isotopic analysis also has the potential as a research tool for organic chlorine compounds, several analytical techniques for isotopes in CAHs have been developed (*2*−*9*). The information about isotope fractionation would be necessary to (a) judge the feasibility of using isotopes to trace the sources of pollutants, (b) evaluate the effectiveness of in-situ bioremediation, and (c) investigate the subsurface process mechanisms. Tanaka and Rye (*2*) reported relatively large Cl isotope variations in the CAHs. In addition, the isotopic compositions of the chlorinated solvents were measured to distinguish contaminants from different manufacturers and to trace them in the environment (*10*). To utilize the unique isotopic compositions of pollutants as a fingerprint of their source, the magnitude of the isotopic fractionation during migration should be quantitatively known.

In-situ cleanup techniques (e.g., bioremediation) would be cost-effective, and they would be applicable for widespread contaminated plumes. However, practical uses of these techniques are limited by the difficulty in proving their effectiveness. In general, the contribution of both biotic and abiotic degradations is not distinguishable from the contribution of other physical processes such as dilution by chemical analysis of the samples obtained from a limited number of sampling points. However, the data for the isotopic variations in contaminated aquifers (e.g., refs *11*−*13*) would complement chemical analysis. Chlorinated ethenes are representative CAHs. In particular, trichloroethene (TCE) and tetrachloroethene (PCE) are among the most serious groundwater contaminants. The reductive dechlorination of chlorinated ethenes mediated by abiotic and biotic systems plays an important role in wastewater treatment, remediation, and natural attenuation of contaminated aquifers and soils (e.g., ref *14*).

In this study, the degree of Cl isotope fractionation during the biotic dechlorination of TCE or PCE to *cis*-1,2-dichloroethene (cDCE) has been investigated in laboratory experiments. Mathematical models for the changes in the isotopic ratios were developed, and we attempted to determine the isotope fractionation factors for each dechlorination step.

Materials and Methods

Microorganisms. Strain T (a sulfate-reducing bacterium isolated from the drainage of a laundry; *15*, *16*), consortium F (an enrichment culture obtained from PCE contaminated soil; *17*), and consortium N (an enrichment culture N-YE obtained from TCE-contaminated soil; *18*, *19*) were used for the biological dechlorination of PCE and TCE. The cultures dechlorinated PCE and TCE to cDCE. The concentrations of the vinyl chloride and *trans*-1,2-dichloroethene in the medium were under the detection limit throughout the reaction. Although, trace 1,1-dichloroethene was detected by GC-FID, it would be negligible against cDCE (strain T, 0.1−0.2%; consortium N and consortium F, 0.1% or less). As a result, the simultaneous reaction of the TCE dechlorination would not affect the following discussions.

The cultures were sustained in 120-mL serum bottles with 50 mL of liquid media containing 10 mg/L of PCE under a N_2 atmosphere. The MMY medium (7 g of K_2HPO_4, 2 g of KH_2PO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, 1 g of $(NH_4)_2SO_4$, 2 g of yeast extract, 0.5 g of trisodium citrate in 1 L of distilled water, pH 7.2; *15*) was used for cultivation of consortium F. For cultivation of strain T, 0.3 g/L of L-cysteine hydrochloride was added to the medium. For cultivation of consortium N, trisodium citrate was omitted from the medium.

Reagents. A reagent-grade TCE (minimum assay 99.5%; Wako Pure Chemical Industries) and PCE (minimum assay 99%; Wako Pure Chemical Industries) were used for the dechlorination experiments. The $\delta^{37}Cl$ values of the neat TCE and PCE were -1.72 ± 0.10‰ and -1.97 ± 0.36‰ ($n = 5$, error: SD), respectively (*9*).

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Chemical Analysis. The concentrations of the chlorinated ethenes in the liquid media were determined by headspace gas analysis using a Shimadzu GC-9A gas chromatograph equipped with a Shimadzu CBP1 Hi-Cap column (0.2 mm i.d. × 25 m, film thickness 0.25 μm) and a flame-ionization detector. As the internal standard, 50 μmol of toluene dissolved in methanol was added to each reaction vessel. The molar fractions of the chlorinated ethenes were the mole amount of each compound normalized with the initial PCE or TCE mole amount.

Dechlorination of Chlorinated Ethenes. The dechlorination experiments were performed in 68-mL serum bottles with 25 mL of the liquid media for consortia F and N. Because strain T could not degrade a high concentration of PCE, the experiments for strain T were performed in 120-mL serum bottles with 50 mL of the liquid medium. The medium (23.8 mL for consortia F or N, 47.5 mL for strain T) was sterilized in an autoclave (121 °C, 15 min). After being cooled, the medium was purged with N_2 gas for 5 min to remove any oxygen in medium and headspace gas, and then 1.2 mL (consortium F or N) or 2.5 mL (strain T) of the subculture was inoculated in the bottle. The purge was continued for 5 min to remove any chlorinated ethenes dissolved in the subculture, and then the bottle was sealed with a poly(tetrafluoroethylene)-lined rubber septum and an aluminum cap. The culture was injected with 10 μL of PCE or TCE stock solution (in methanol) containing 10 μmol of PCE or TCE and then incubated at 30 °C. The progress of the reaction was monitored by headspace analysis by GC-FID. Toluene (3 mL) was added through the septum to stop the reaction. The bottle was then shaken vigorously to extract the chlorinated ethenes including the PCE, TCE, and dechlorination products from the medium and headspace. After the toluene layer was moved to a test tube, 2 mL of toluene was added to the bottle, and the remaining chlorinated ethenes were recovered. Then, those toluene layers were combined. More than 7 bottles were prepared for a series of dechlorination experiments, one of which was sacrificed for extraction at each reaction stage.

Isotopic Analysis of Chloroethenes. For the isotopic measurement of Cl by thermal ionization mass spectrometry, the organic chlorine was converted to CsCl (*9*, *20*). The toluene layers that contained the chlorinate ethenes were dried using Na_2SO_4. A 0.4-mL aliquot of sodium biphenyl reagent (1 M solution in dimethoxyethane; Dojindo Laboratory) was then added to the solution. The liberated Cl^- was extracted with water and 1 M HNO_3. The extracted fractions containing Cl^- were combined, and a portion of the solution that contained 50 μg of Cl^- was transferred to a polypropylene centrifuge tube. A solution of $AgNO_3$ was added to the tube, and the obtained AgCl precipitation was dissolved in 1 M NH_3. To remove the Ag ion, Mg powder was added to the solution. The obtained supernatant was recovered and evaporated to dryness on a hot plate (120 °C). The residue ($MgCl_2 + NH_4Cl$) was dissolved in water and loaded on a column that contained 0.1 mL of a Cs^+-form cation-exchange resin prepared from DOWEX 50Wx8 (H^+-form, 200−400 mesh) and an aqueous solution of Cs_2CO_3. The eluent containing CsCl was treated with activated charcoal powder (GL Sciences GX-60) because organic contaminants would prevent thermal ionization of CsCl to Cs_2Cl^+. The supernatant was evaporated to dryness on a hot plate. Water was then added to the residue (CsCl) to form a 2 g of Cl/L solution.

Isotopic determinations were performed using a Finnigan MAT 262 thermal ionization mass spectrometer (*9*, *20*). Two micrograms of chlorine as a CsCl aqueous solution and ca. 70 μg of graphite powder (Nilaco) as a slurry in 80% ethanol were mixed. The mixture was loaded on the center of a Ta filament (width: 1.5 mm; thickness: 0.025 mm; Nilaco) and then dried. The filaments were loaded in the ion source of the mass spectrometer. After evacuation of the ion source, the intensity of the $Cs_2{}^{35}Cl^+$ ion current was adjusted to (2−3) × 10^{-13} A by the filament current, which was typically 1.4−1.5 A. Data (ratio of ion current of $Cs_2{}^{37}Cl^+$ and $Cs_2{}^{35}Cl^+$ as $^{37}Cl/^{35}Cl$) were acquired in the static (multi-Faraday cup) mode for less than 100 min. The typical reproducibility of the isotopic ratio measurements was 0.2−0.4‰ (RSD).

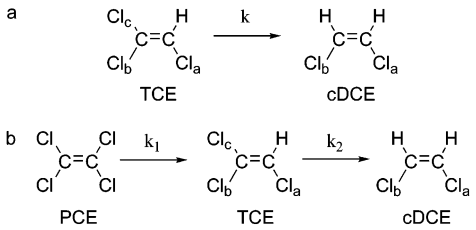

FIGURE 1. Dechlorination of (a) TCE and (b) PCE to cDCE by anaerobic bacteria.

Because the chlorinated ethenes were not separated, all data shown in this study are the Cl isotopic compositions for the bulk chlorinated ethenes (PCE, TCE, and cDCE). The isotopic compositions of chlorine are expressed in per mil deviation from that of the standard mean ocean chloride (*21*). Actually, reagent-grade CsCl from Nacalai Tesque, Inc. was used as a routine laboratory standard of the Cl isotopic composition (*20*) during the course of this study in order to correct any possible offset caused by a change in the instrumental configuration (*22*). One or two standards were normally loaded into the mass spectrometer together with the samples, and the δ values of the samples were calculated from

$$\delta^{37}\mathrm{Cl}_{\mathrm{SMOC}}\,(‰) = \left[\frac{R_{\mathrm{sample}}(1 + 10^{-3}\delta_{\mathrm{CsCl}})}{\bar{R}_{\mathrm{CsCl}}} - 1\right] \times 1000 \quad R = \frac{^{37}\mathrm{Cl}}{^{35}\mathrm{Cl}} \quad (1)$$

where δ_{CsCl} is −2.49 (per mil deviation of isotopic ratios of the CsCl reagent from seawater), and $\bar{R}$ is the average of standard CsCl isotopic ratios in the sample analysis period.

Isotopic Fractionation Model for TCE Dechlorination. The biotic reductive dechlorination of TCE is a regioselective reaction (Figure 1a). Only the Cl_c is substituted with a hydrogen atom, and cDCE is produced. Thus, we assumed that the isotopic compositions of the Cl_a and the Cl_b would remain constant during the dechlorination. On the other hand, the isotopic composition of the Cl_c would change during the process. The isotope ratio of the bulk organic chlorine (assuming only TCE and cDCE exist) is represented as a binary mixing relationship between R_2 (isotopic ratio in $Cl_a + Cl_b$) and R (isotopic ratio in Cl_c):

$$R_t = \frac{N_c R(1 + R_2) + (1 - N_c)R_2(1 + R)}{N_c(1 + R_2) + (1 - N_c)(1 + R)} \quad (2)$$

where R_t is the Cl isotopic ratio of organic Cl (TCE + cDCE), N_c is the molar fraction of Cl_c.

When the change in the isotopic ratio is small enough, the ratio of $(1 + R_2)$ and $(1 + R)$ approach unity. Thus eq 2 reduced to

$$R_t \cong N_c R + (1 - N_c)R_{ab} \quad (3)$$

Using the molar fraction of the remaining TCE (f_T), N_c becomes

$$N_c = \frac{f_T}{f_T + 2} \quad (4)$$

If the isotopic fractionation of Cl_c during the dechlorination follows Rayleigh equation:

$$R = R_1 f_T^{(\alpha-1)} \quad (5)$$

where R_1 is the initial isotope ratio of Cl_c, and α is the fractionation factor ($\alpha = 1 + \epsilon/1000$; ϵ is the enrichment factor). Substituting eqs 4 and 5 into eq 3 gives

$$R_t = \frac{R_1 f_T^{\alpha} + 2R_2}{f_T + 2} \quad (6)$$

Isotopic Fractionation Model for PCE Dechlorination. The dechlorination of PCE to cDCE (Figure 1b) will be regarded as a sequential first-order reaction (*23*). Theoretically, changes in the chloroethene concentrations with time are represented as follows (f_P, f_T, and f_D are the fractions of PCE, TCE, and cDCE, respectively):

$$f_P = e^{-k_1 t} \quad (7)$$

$$f_T = \frac{k_1}{k_1 - k_2}(e^{-k_2 t} - e^{-k_1 t}) \quad (8)$$

$$f_D = 1 - f_P - f_T \quad (9)$$

Because the four Cl atoms in the PCE molecule are equivalent, one-fourth of the Cl atoms may be randomly eliminated during the first step of the PCE dechlorination. If the dechlorination of TCE does not occur, the isotopic ratio change of Cl in PCE and TCE will be represented as follows using the Rayleigh equation:

$$R_{PTD}(f_P) = R_0\{(3 + f_P)/4\}^{(\alpha_1-1)} \quad (10)$$

where α_1 is the isotope fractionation factor for the first dechlorination step.

During the second dechlorination step, Cl_c in TCE molecule would be eliminated, and Cl_a and Cl_b would remain in the cDCE molecule. Namely, the total isotope composition of $Cl_a + Cl_b$ in the TCE molecule and Cl in the PCE and cDCE molecules will change as in eq 10. Meanwhile, the isotopes of Cl_c in TCE might be further fractionated. A fractionation model of Cl_c was calculated from the material balance of ^{37}Cl in the system and eqs 7−10. The obtained differential equation was numerically solved using the commercially available software MATLAB (Math Works). The change in the isotope ratio of the total organic chlorine (assuming only PCE, TCE, and cDCE exist) was then calculated as a binary mixing equation with a numerical solution of the differential equation and isotopic composition of the other Cl atoms (eq 10).

Results

Isotope Fractionation Associated with TCE Dechlorination. The mathematical model of the Cl isotopic shift (eq 6) plotted versus the fraction of remaining TCE is shown in Figure 2. The isotopic composition of the bulk organic chlorine (Cl in TCE and cDCE) is given by the weighted mean of the isotopic compositions of Cl_c and $Cl_a + Cl_b$. Because of the preferential elimination of ^{35}Cl, the Cl isotopic ratio ($^{37}Cl/^{35}Cl$) of the residual Cl_c would increase following the Rayleigh equation. During the first stage of the reaction, the isotopic ratio in the bulk organic chlorine would increase because this mass discrimination effect is dominant. As the reaction progressed, the amount of Cl_c and its contribution to the bulk Cl isotopic composition would decrease. As a consequence, the isotopic ratio would decrease and reach the isotopic composition of $Cl_a + Cl_b$ at the end of the reaction. The smaller isotope fractionation factor (i.e., the larger isotope effect) would produce a larger curvature in the trajectories.

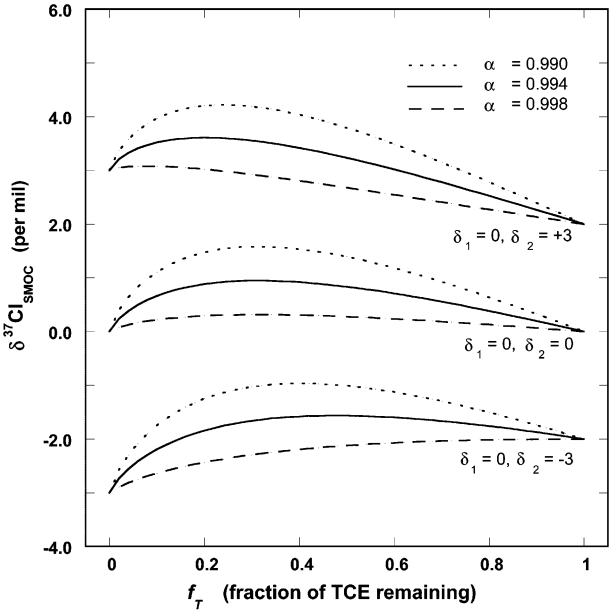

FIGURE 2. Calculated trajectories of $\delta^{37}Cl$ value of organic chlorine (TCE and cDCE) vs fraction of remaining TCE for different parameters. δ_1 and δ_2 represent initial $\delta^{37}Cl$ value of Cl_c and $Cl_a + Cl_b$, respectively. α represents isotope fractionation factor.

The results of the isotopic measurements during the biotic TCE dechlorination by three kinds of anaerobic bacteria culture are shown in Figure 3. The changes of the isotopic composition during the biotic dechlorination were small, but the depletion trend in ^{37}Cl during the later stage of dechlorination was observed in all cases. The difference in the isotopic ratio trajectories was not significant because of the relatively large errors in the isotopic analysis. A least-squares regression of the data shown in Figure 3 yields the best fit α, δ_1, and δ_2 as follows for the three bacterial cultures utilizing eq 6; strain T: $\alpha = 0.9945 \pm 0.0009$, $\delta_1 = -0.53 \pm 0.33‰$, $\delta_2 = -2.22 \pm 0.13‰$; consortium N: $\alpha = 0.9944 \pm 0.0007$, $\delta_1 = -0.22 \pm 0.29‰$, $\delta_2 = -2.44 \pm 0.12‰$; consortium F: $\alpha = 0.9943 \pm 0.0010$, $\delta_1 = -0.16 \pm 0.44‰$, $\delta_2 = -2.38 \pm 0.16‰$.

Isotope Fractionation Associated with PCE Dechlorination. For the PCE dechlorination, features of the time courses of the PCE, TCE, and cDCE concentrations corresponded to the theoretical sequential first-order reaction except for the existence of the lag phase (*24*). The mathematical model for the Cl isotopic change plotted versus the fraction of PCE remaining is shown in Figures 4 and 5. The isotopic shift is controlled by the isotope fractionation factors α_1 (PCE dechlorination) and α_2 (TCE dechlorination) (Figure 4) and ratio of the rate constant of the reactions, k_2/k_1 (Figure 5). Mainly the α_1 value would control the change in the isotopic ratio during the dechlorination. Because the α_2 value and k_2/k_1 are related to the intermediate TCE, the effect of these values on the trajectories is small. A lower α_2 value (i.e., larger isotopic effect) would give a higher Cl isotopic ratio in the TCE as shown in the previous section. The fraction of TCE can be expressed using the fraction of PCE as the following equation that was led from eqs 7 and 8:

$$f_T = \frac{1}{k_2/k_1 - 1}(f_P - f_P^{k_2/k_1}) \quad (11)$$

The maximum of the TCE fraction increases as the k_2/k_1 ratio decreases (i.e., the relative reaction rate of TCE

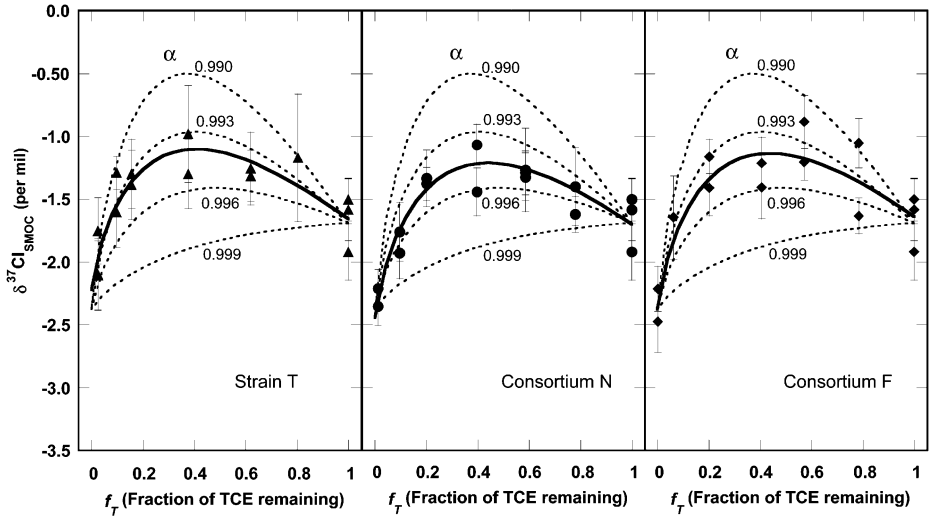

FIGURE 3. δ^{37}Cl value of organic chlorine (TCE and cDCE) vs fraction of remaining TCE from the TCE dechlorination experiments. Error bars represent the internal error of each run of the Cl isotopic measurement ($2\sigma_m$). Dotted lines are calculated trajectories of δ^{37}Cl value of organic chlorine (eq 6) for different α values. δ_1 and δ_2 for the trajectories are the average of δ_1 and δ_2 in three regressions (δ_1 and δ_2 represent initial δ^{37}Cl value of Cl_c and δ^{37}Cl value of $Cl_a + Cl_b$, respectively).

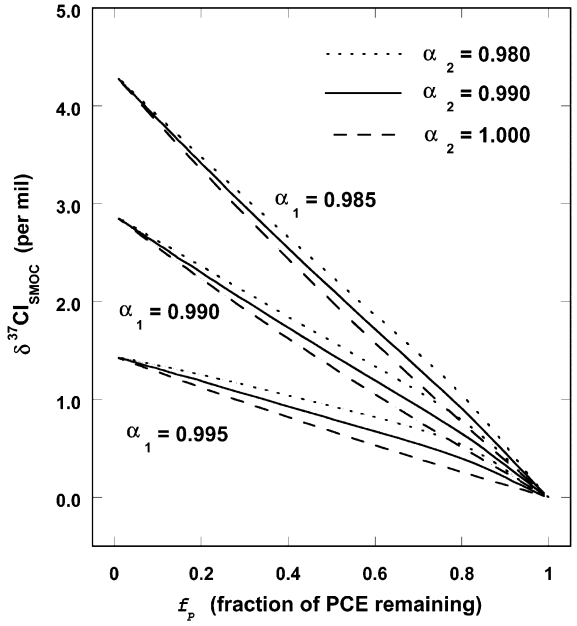

FIGURE 4. Calculated δ^{37}Cl value trajectories of organic chlorine (PCE, TCE, and cDCE) vs fraction of PCE for different values of isotope fractionation factors with constant k_2/k_1 value. δ_0 represents initial δ^{37}Cl value of PCE (0‰). α_1 and α_2 represent isotope fractionation factor for PCE and TCE dechlorination, respectively. k_2/k_1 represents ratio of rate constants of TCE dechlorination and PCE dechlorination ($k_2/k_1 = 15$).

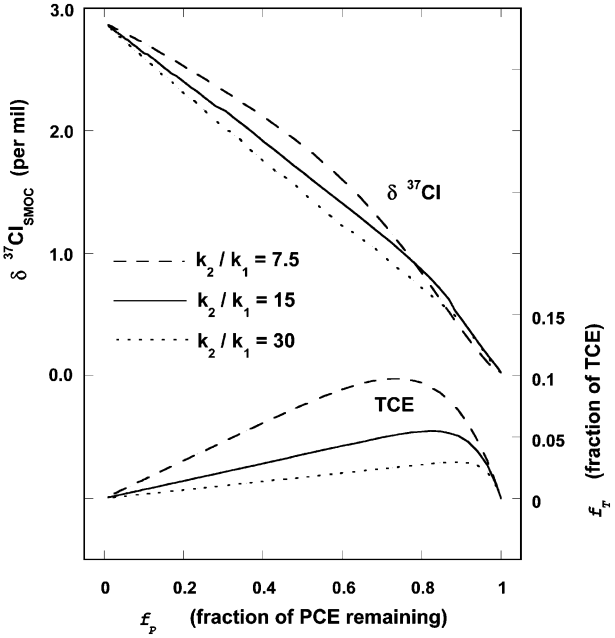

FIGURE 5. Calculated δ^{37}Cl value trajectories of organic chlorine (PCE, TCE, and cDCE) and fraction of TCE vs fraction of PCE for different k_2/k_1 values with constant values of isotope fractionation factors. The trajectories of TCE fraction were calculated by eq 11. δ_0 represents initial δ^{37}Cl value of PCE (0‰). α_1 and α_2 represent isotopic fractionation factor for PCE and TCE dechlorination, respectively ($\alpha_1 = 0.990$, $\alpha_2 = 0.980$). k_2/k_1 represents ratio of rate constants of TCE and PCE dechlorination.

dechlorination decreases). These are the reasons why the isotopic ratio trajectories shift upward as the α_2 and k_2/k_1 values decrease.

Compared with the TCE dechlorination, the actual changes in the Cl isotopic compositions during biological PCE dechlorination were greater (Figure 6). The general trends toward higher δ^{37}Cl values with the PCE degradation were consistent with the theoretical models (dotted lines in Figure 6), and the upward shift of the curves was slightly greater when the k_2/k_1 ratio was smaller. The values of α_1 were 0.987−0.991 as rough estimates from the δ^{37}Cl values at the initial and final reaction stage. As observed in Figure 6, the δ^{37}Cl values and their errors span a large range of potential fractionation factor values (calculated lines of α_2 between 0.970 and 1.000 can all be made to fit the data). Clearly, the precision of the measurements was not high enough to determine the values of α_2. The results including the rate constants and the isotope fractionation factors for the dechlorination reactions are summarized in Table 1.

Discussion

Highly chlorinated organic compounds are usually nonbiodegradable by aerobes. Moreover, the supply of electron acceptors, especially molecular oxygen, is the limiting factor for the in-situ biodegradation of organic pollutants in subsurface environments. As a result, microbial communities

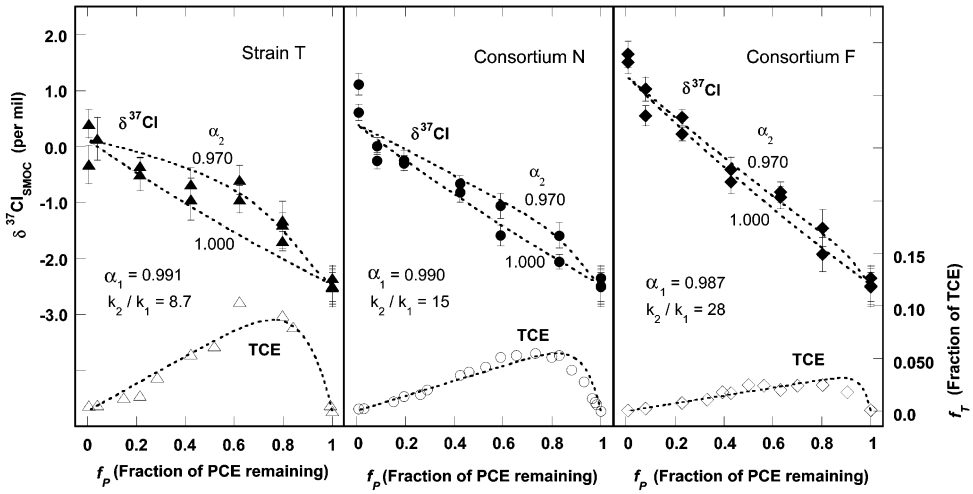

FIGURE 6. δ^{37}Cl value of organic chlorine (PCE, TCE, and cDCE) and fraction of TCE vs fraction of PCE remaining from the PCE dechlorination experiments. Dotted lines are calculated trajectories of δ^{37}Cl value of organic chlorine and fraction of TCE. Error bars represent the internal error of each run of Cl isotopic measurement ($2\sigma_m$). δ_0 for calculated trajectories is average of initial δ^{37}Cl value of PCE (−2.47‰). α_1 and α_2 represent isotopic fractionation factor for PCE and TCE dechlorination, respectively. k_2/k_1 represents ratio of rate constants of TCE and PCE dechlorination. The theoretical trajectories of TCE fraction were calculated by eq 11.

TABLE 1. Properties of Anaerobic Bacteria Used for the Biotic Dechlorination Experiments

	strain T	consortium N	consortium F
nitrate reduction	nd[a]	nd	+
sulfate reduction	+	−	nd
methanogenesis	−	−	−
	PCE → cDCE		
k_1(PCE→TCE)	0.061 h^{-1}	0.11 h^{-1}	0.12 h^{-1}
k_2(TCE→cDCE)	0.53 h^{-1}	1.7 h^{-1}	3.4 h^{-1}
α_1(PCE→TCE)	0.991	0.990	0.987
	TCE → cDCE		
k(TCE→cDCE)	0.16 h^{-1}	0.14 h^{-1}	0.25 h^{-1}
α(TCE→cDCE)	0.9945 ± 0.0009	0.9944 ± 0.0007	0.9943 ± 0.0010

[a] nd, not determined.

and isolates that can reductively dechlorinate high concentrations of chlorinated organic compounds under anaerobic conditions have a potential for bioaugmentation and natural attenuation (e.g., ref *25*). The number of enriched culture of anaerobic bacteria that can dechlorinate PCE and TCE have been reported, and there is on going research on isolated microorganisms and molecular biological studies of dechlorination enzyme systems (e.g., ref *26*).

The isotopic data of chlorinated organic compounds can be used to constrain the degradation mechanisms in subsurface environments (*6*, *11*−*14*, *27*−*33*). It might be possible to evaluate the contribution of biotic or abiotic degradation to the attenuation of pollutants and to identify microorganisms (and their metabolic pathways) mediating the degradation of pollutants by using quantitative information on isotope effects in various subsurface processes. Although GC/C/IRMS is a powerful tool to trace natural or artificial organic compounds by compound-specific δ^{13}C or δ^{15}N fingerprints (e.g., ref *12*), such on-line isotopic ratio mass spectrometry for Cl has never been developed. As a consequence, the application of Cl isotope data for the environmental issues is difficult under the present condition.

The most important and basic parameter of isotope chemistry, the fractionation factor, is usually estimated from the isotopic ratio change using the Rayleigh equation (*34*). For example, the Cl isotopic fractionation during aerobic degradation of dichloromethane by methylotroph is expressed in a simple Rayleigh model (*28*). However, it is difficult to apply such a model to the data obtained from other multistep reactions without any modification (e.g., dechlorination of chlorinated ethenes by anaerobes (*12*, *14*, *32*), bacterial reduction of selenate to elemental selenium via selenite (*35*)). Therefore, the relatively simple one-step process (TCE to cDCE) and the two-step process (PCE to cDCE) were investigated in this study. We have developed the mathematical models to determine the isotopic fractionation factors of the dechlorination reactions using non-compound-specific Cl isotope data. Although the actual changes in the isotope compositions were roughly consistent with the models (Figure 3 and 6), more precise and/or compound-specific isotopic measurement techniques should be developed to interpret the small isotopic change like the TCE dechlorination case. Although an isotopic change in the eliminated Cl^- may complement the organic chlorine data, the isotopic analysis of Cl^- was impossible because of the high concentration of organic compounds and background Cl^- in the media.

A few studies have been published on Cl isotope fractionation during the biotic and abiotic reactions of chlorinated organic compounds. The isotope fractionation factors for abiotic dechlorination (solvolysis, reductive dehalogenation, and dehydrodechlorination) range from 0.991 to 0.996 (*36*−*39*). Heraty et al. (*28*) reported the isotopic fractionation factor for the aerobic biodegradation of dichloromethane (α = 0.9962). The factors for the hydrolytic dechlorination of 1,2-dichloroethane and 1-chlorobutane with a haloalkane dehalogenase were 0.996 and 0.9934, respectively (*40*). There is no significant difference between the fractionation factors obtained in this study and those reported values.

Further studies on factors that may possibly affect isotopic fractionation during the dechlorination of chlorinated ethenes will be necessary to make the models more precise. In this study, the possibilities of isotope exchange between chlorinated ethenes and Cl^- in the media (*41*, *42*) and rearrangement of Cl atoms in the molecules were neglected during the development of the mathematical models. Moreover, the bacteria population change in consortiums (*43*) and the rate constant and isotope fractionation factor change depending on the growth stage of the bacteria would affect the trajectories of the isotopic composition.

The Cl isotopic composition of Cl_c in the TCE reagent was significantly different from the composition of $Cl_a + Cl_b$. Such intramolecular distributions of isotopes constrain the be-

havior of substances more specifically because a difference in the reactivity of the atoms on each site in asymmetric molecules would reflect the intramolecular site-specific isotope ratio changes during the synthesis or degradation processes (*39*, *44*).

The analytical techniques and the mathematical models developed in this study are preliminary steps to apply isotopic data to the evaluation of the possible disturbance on tracing pollution sources by their isotopic fingerprint, quantification of in-situ bioremediation effectiveness, and investigation of mechanisms in subsurface processes.

Acknowledgments

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Supporting Information Available

Isotopic fractionation model for PCE to cDCE declorination. This material is available free of charge via the Internet at http://pubs.acs.org.

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