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Stable Carbon Isotope Fractionation of Chloroethenes by Dehalorespiring Isolates

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Stable carbon isotope fractionation during the reductive dechlorination of chloroethenes by two bacterial strains that dechlorinate to ethene, *Dehalococcoides ethenogenes* 195 and *Dehalococcoides* sp. strain BAV1 as well as *Sulfurospirillum multivorans* and *Dehalobacter restrictus* strain PER-K23, isolates that do not dechlorinate past DCE, are reported. Fractionation by a *Dehalococcoides*-containing enrichment culture is also measured for comparison to the isolates. All data adequately fit the Rayleigh model and results are presented as enrichment factors. For strain 195, the measured enrichment factors were -9.6 ± 0.4, -21.1 ± 1.8, and -5.8 ± 0.5 when degrading TCE, cDCE, and 1,1-DCE, respectively. Strain BAV1 exhibited enrichment factors of -16.9 ± 1.4, -8.4 ± 0.3, -21.4 ± 0.9, and -24.0 ± 2.0 for cDCE, 1,1-DCE, tDCE, and VC, respectively. The surprisingly large differences in enrichment factors caused by individual reductases (RDases) reducing different chloroethenes is likely the result of chemical structure differences among the chloroethenes. For TCE reduction, *S. multivorans* and *D. restrictus* strain PER-K23 exhibited enrichment factors of -16.4 ± 1.5 and -3.3 ± 0.3, respectively. While all of the organisms studied here utilize RDases that require corrinoid cofactors, the biotic TCE enrichment factors varied widely from those reported for the abiotic cobalamin-catalyzed reaction, indicating that additional factors affect the extent of fractionation in these biological systems. The enrichment factors measured for the *Dehalococcoides*-containing enrichment culture did not match well with those from any of the isolates, demonstrating the inherent difficulties in predicting fractionation factors of undefined communities. Although compound-specific isotope fractionation is a powerful tool for evaluating the progress of in situ bioremediation in the field, given the wide range of enrichment factors associated with functionally similar and phylogenetically diverse organisms, caution must be exercised when applying enrichment factors for the interpretation of dechlorination data.

Introduction

Chloroethenes such as tetrachloroethene (PCE), trichloroethene (TCE), isomers of dichloroethene (DCE), and vinyl chloride (VC) are common groundwater pollutants in the U.S. due to their improper disposal and storage (*1*). These compounds pose a serious health threat due to their toxicity and potential carcinogenicity (*2*). Although in situ bioremediation has recently become a favorable alternative to remediate these compounds at contaminated sites (*3*, *4*), the assessment of the progress and success of this technology in heterogeneous field environments remains a challenging task. Biological reactions in a subsurface aquifer are often accompanied and masked by transport processes such as dissolution, volatilization, and sorption, making it difficult to attribute changes in chloroethene concentration to biological conversion. One promising solution to overcome this problem is the quantification of shifts in the stable carbon isotope ratios of the chloroethenes to evaluate the extent of biodegradation (*5*, *6*). Previous research has shown that physical processes cause minor or negligible shifts in isotope ratios (*7*−*10*), while biological conversion can cause significant changes in the isotope ratios of chloroethenes (*11*−*17*).

For the reductive dechlorination of chloroethenes, bacteria within the genus *Dehalococcoides* are the only organisms that have been shown to metabolically reduce PCE and TCE to the innocuous product ethene (*18*). Within the *Dehalococcoides* genus, different strains have been found to contain different reductive dehalogenase (RDase) genes that catalyze the dechlorination reaction. For example, *Dehalococcoides ethenogenes* 195 (*19*) and *Dehalococcoides* sp. strain FL2 (*20*) contain the *tceA* gene, while *Dehalococcoides* spp. strains VS and GT contain the *vcrA* gene (*21*, *22*), and *Dehalococcoides* sp. strain BAV1 contains the *bvcA* gene (*23*). As reflected in their RDase gene variability, these strains have different capabilities to metabolize or co-metabolize different chloroethenes (summarized in ref *21*). In addition to the three aforementioned genes whose functions have been characterized, annotation of the genomes of strains 195 and CBDB1 (*19*, *24*) together with other molecular studies (*23*, *25*, *26*) have revealed the presence of a large number of putative RDase genes in *Dehalococcoides* spp. that could also play a functional role in dechlorination.

Organisms that dechlorinate PCE and TCE but cannot dechlorinate past DCE come from diverse genera and a review of these organisms is given by Smidt and de Vos (*18*). Interestingly, despite significant physiological and phylogenetic differences among dehalorespiring organisms, the biochemical and molecular characteristics of the RDases do share similar characteristics. On the biochemical level, the majority of the enzymes are recognized to contain two iron−sulfur clusters as cofactors and are corrinoid-dependent (*18*, *27*−*30*). Corrinoids are four-pyrrole ring structures that form the basis for compounds such as vitamin B-12 or cyanocobalamin (*31*). Amino acid analysis has revealed conserved motifs such as the twin arginine signal sequence for translocation of the catalytic protein across the cytoplasmic membrane and the presence of a membrane anchoring protein for the catalytic subunit (*18*, *29*, *30*).

Biological transformations cause shifts in the isotopic ratio of compounds because of the stronger and less reactive molecular bonds formed by heavier isotopes in comparison to lighter isotopes. Hence, reaction rates tend to be faster for molecules with light isotopes than for molecules with heavy isotopes, resulting in enrichment of the heavy isotopes in the residual reactant and formation of daughter products that are enriched with the light isotope. These shifts are referred to as kinetic isotope fractionation (*32*) and can be quantified using compound-specific stable isotope analysis. The magnitude of the fractionation in a microbial process

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is a function of the microorganisms, the enzymes and reaction mechanisms, and the compounds being transformed. Compound-specific stable isotope analysis can be used to qualitatively demonstrate in situ biological activity and to quantitatively estimate the extent of biodegradation (*33*–*36*).

A number of laboratory studies (*11*, *13*, *37*, *38*) have applied compound-specific carbon isotope analysis to measure kinetic isotope fractionation during reductive dechlorination. The magnitude of the isotopic fractionation effects observed for these studies were highly variable. A majority of these studies were performed with mixed cultures enriched from field samples, raising the possibility that different organisms utilizing different pathways to degrade chloroethene compounds may cause different isotopic shifts. In this study, the stable carbon isotope fractionation caused by reductive dechlorination of chloroethenes by two pure cultures of *Dehalococcoides*, *D. ethenogenes* 195 (*39*) and strain BAV1 (*40*), along with a well-characterized *Dehalococcoides*-containing enrichment (*41*, *42*), and two isolates that cannot dechlorinate past DCE, *Sulfurospirillum multivorans* (*43*, *44*) and *Dehalobacter restrictus* strain PER-K23 (*45*) has been studied.

Materials and Methods

Chemicals. All chloroethenes were purchased from Fisher Scientific (Houston, TX), Sigma-Aldrich (St. Louis, MO), or Fluka (Seelze, Germany) at the highest purity available. The same bottle or cylinder of chemical was used throughout the study to minimize variability in the starting isotope ratio.

Culture Sources and Growth Conditions. Pure cultures of *Dehalococcoides ethenogenes* 195 and *Dehalococcoides* sp. BAV1 were kindly provided by Dr. Stephen Zinder of Cornell University and Dr. Frank Löffler of the Georgia Institute of Technology, respectively. *S. multivorans* (DSM 12446) and *D. restrictus* strain PER-K23 (DSM 9455) were purchased from Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH (Braunschweig, Germany). All isolates were grown in 160 mL serum bottles with 100 mL of media as described previously (*46*). Acetate was amended at 5 mM as the carbon source, the headspace was filled with H_2/CO_2 (80:20) as the electron donor source, and one to three doses of 56 μmol of TCE were added as the terminal electron acceptor. *D. restrictus* strain PER-K23 was the only culture that received 0.1 g/L peptone. Isolates were routinely transferred at 3% (v/v) to fresh medium after all electron acceptors had been reduced. The *Dehalococcoides*-containing enrichment culture used in this study (designated ANAS) has been stable in our laboratory for 7 years, and its growth, maintenance, and molecular characterization have been described elsewhere (*41*, *42*).

Isotope Fractionation Experimental Setup. All experiments were conducted in 160 mL serum bottles with 100 mL of media as described above. Between 60 to 70 μmol of the appropriate chloroethene (TCE, *cis*-1,2-DCE (cDCE), *trans*-1,2-DCE (tDCE), 1,1-DCE, or VC) was added as a gas or pure-phase liquid to each serum bottle 42 h prior to inoculation to facilitate equilibration and ensure accurate sampling at time zero. Isolates only received chloroethenes that they have been previously reported to be able to use as metabolic electron acceptors. For experiments with isolates, 3% (v/v) of a previously grown culture was inoculated at time zero. For experiments with ANAS, 10% (v/v) of inoculum, 50 mM lactate, and a N_2/CO_2 (90:10) headspace were used. Strain 195 and *D. restrictus* strain PER-K23 were incubated at 34 °C, and the rest were at 30 °C. All experiments were conducted with duplicate biotic bottles and one abiotic bottle with the same volume of sterile media added in place of the culture inoculum.

At each sampling time point, 100 μL of headspace was taken for immediate measurement of chloroethene and ethene concentrations. For isotope analysis, 7 mL of liquid was withdrawn from each experimental bottle and stored in a previously prepared 10 mL vial that was capped with a blue butyl stopper. An equal volume of the appropriate headspace gas was injected back into the bottle to prevent formation of a vacuum. Vials for isotope samples contained 1 mL of 1 M NaOH to kill the cells and 1.1 g of NaCl to facilitate volatilization of chloroethene into the headspace by increasing the solution ionic strength (*10*). pH papers were used to confirm >12 pH, and the vials were stored in the dark at 4 °C prior to analysis.

Analytical Methods. Concentrations of chloroethenes and ethene were measured by injecting 100 μL of headspace gas into an HP 5890 gas chromatograph equipped with a flame ionization detector (GC-FID) as described previously (*47*). External calibration curves were generated using serum bottles containing anaerobic medium with known amounts of each compound. Headspace concentrations (C_G) were converted to total mass of solvents (M) in vials using Henry's constant (H) (*48*, *49*) according to a material balance

$$M = C_G V_G + \frac{C_G}{H} V_L \quad (1)$$

where V_G and V_L are the gas and liquid volumes in the vials, respectively. The total amount of mass loss due to sampling for solvent concentration was negligible. Mass loss due to removal of liquid for isotope analysis was calculated based on the liquid concentration at each time point and was corrected for in the enrichment factor calculation.

For stable carbon isotope analysis, 300−1000 μL of headspace sample was taken from the sample vial and injected into a gas chromatograph-combustion-isotope ratio mass spectrometry system (GC-C-IRMS) as described previously (*15*). Briefly, the GC-C-IRMS system consisted of a Hewlett-Packard 6890 gas chromatograph that was fitted with a Supelco Supel-Q-Plot capillary column (0.32 mm × 60 m) for the separation of chloroethenes, a Micromass combustion interface at 850 °C, and a Micromass JA Series Isoprime isotope ratio mass spectrometer (Micromass, Manchester, U.K.). Prior to injection into the GC-C-IRMS, the headspace sample was cryogenically trapped in a stainless steel loop submerged in liquid nitrogen that was connected to a six-port valve in line on the gas chromatograph. Once the valve was activated, the liquid nitrogen was then removed, the loop was defrosted with a heat gun, and the cryogenically trapped sample was carried by a helium gas stream into the GC-C-IRMS. Oven settings for chloroethene separation varied depending on the compounds being analyzed. For TCE and DCE isomers, the initial temperature was 80 °C for 1 min, ramped to 180 °C at 20 °C/min, and then held for varying length according to the elution time of the compounds. For VC, the initial temperature was 40 °C for 1 min, followed by a ramp of 10 °C/min to 80 °C, then 20 °C/min to 180 °C. Carbon isotope ratios ($R = {}^{13}C/{}^{12}C$) are expressed in the conventional δ notation and reported in per mil (‰)

$$\delta^{13}C\ ((‰)) = ((R_{sample}/R_{standard}) - 1)1000 \quad (2)$$

where $R_{sample}/R_{standard}$ are carbon isotope ratios for the sample and Vienna Pee Dee Belemnite (VPDB) standard, respectively. The reference CO_2 gas standard for the GC-C-IRMS was calibrated using a dual-inlet mass spectrometer (VG Prism series II). On the basis of repeated analyses of laboratory standards, the uncertainty associated with the isotope measurement was ±0.5‰ (2σ).

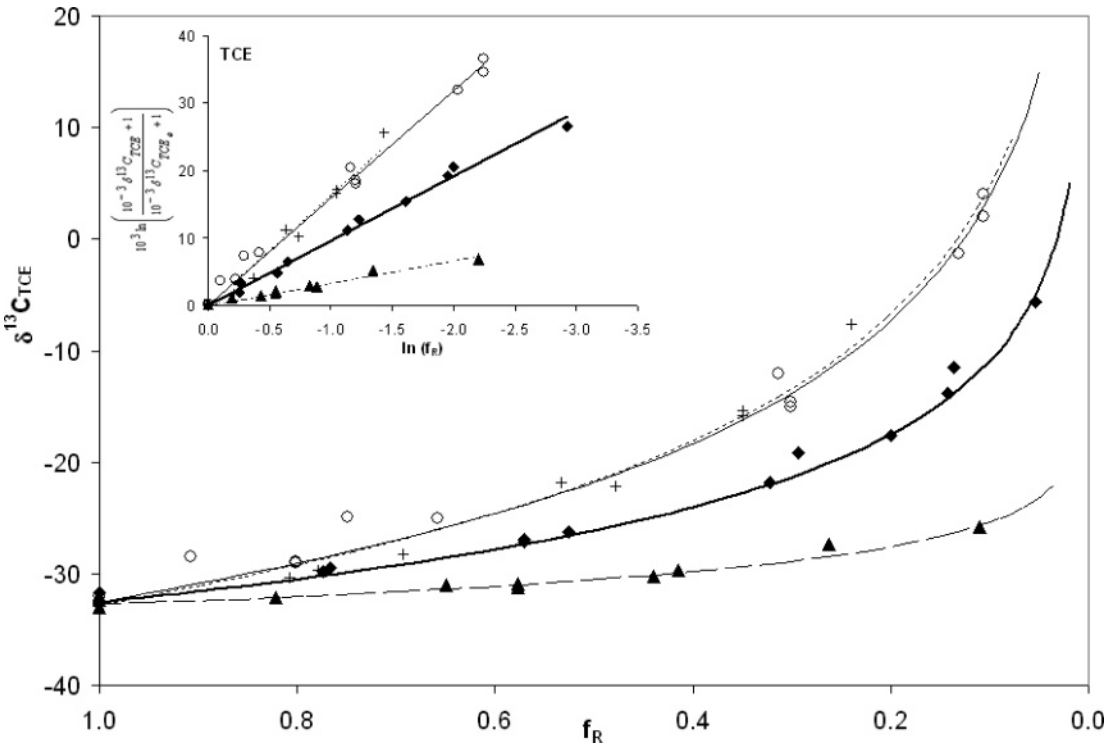

FIGURE 1. Stable carbon isotope fractionation of TCE by different dechlorinating cultures. Symbols represent measurements from duplicate experimental bottles, and lines are fitted to the Rayleigh model using the calculated ϵ. The inserted graph shows the Rayleigh equation linear regression plot. (◆) and (—) denote strain 195; (+) and (···) *S. multivorans*; (▲) and (– –) *D. restrictus* strain PER-K23; and (○) and (–) ANAS.

Calculation of Enrichment Factors. The isotope shifts during the reductive dechlorination of chloroethenes were described in terms of an enrichment factor (ϵ) according to the Rayleigh model (*50*)

$$10^3 \ln\left(\frac{10^{-3}\delta^{13}C_R + 1}{10^{-3}\delta^{13}C_{R0} + 1}\right) = \epsilon \ln(f_R) \quad (3)$$

where f_R is the fraction of reactant remaining as determined according to the total mass measured at time zero and at each time point in a closed system, and $\delta^{13}C_{R0}$ and $\delta^{13}C_R$ are the isotope ratios of the initial and remaining reactant, respectively. The enrichment factor was calculated by plotting the left side of eq 3 against $\ln(f_R)$, and a linear regression with a *y*-intercept of zero was applied to determine the slope (ϵ) and 95% confidence interval (CI). Because the Rayleigh model is only valid for an irreversible reaction of a homogeneous reactant, the enrichment factors calculated using eq 3 were based on the mass and isotope changes of the initially amended chloroethene only. Although daughter products were quantified to ensure complete mass balances, fractionation of the daughter products was not considered in the calculation as simultaneous production and dechlorination precludes application of the Rayleigh equation.

Results

Isotope Fractionation during TCE Dechlorination. The carbon isotope fractionation caused by the reductive dechlorination of TCE to cDCE by *D. ethenogenes* 195, *S. multivorans*, *D. restrictus* strain PER-K23, and the ANAS enrichment culture is shown in Figure 1. As expected, the isotope ratio of the residual TCE increased as the transformation proceeded, reflecting an enrichment in ^{13}C relative to ^{12}C. *D. ethenogenes* 195 degraded over 90% of the amended TCE, and the isotope composition changed from $\delta^{13}C = -31.6$ to -5.6‰, a fractionation of over 26.0‰. A slightly greater fractionation was observed in the *Dehalococcoides*-containing enrichment ANAS as TCE fractionated by 36.1‰ during 90% degradation. For the two isolates that cannot dechlorinate past cDCE, *S. multivorans* exhibited a fractionation similar to the *Dehalococcoides* spp. as the TCE changed by 25.1‰ during 75% degradation. In contrast, *D. restrictus* strain PER-K23 showed the smallest fractionation with a change of only 6.6‰ over 90% degradation.

As shown in Figure 1 and Table 1, the Rayleigh equation produced good fits to the TCE transformation data with linear regression $r^2 > 0.97$. The Rayleigh enrichment factors ranged from -3.3 ± 0.3 to -16.4 ± 1.5‰, with the least negative enrichment factor exhibited by *D. restrictus* strain PER-K23 and the most by *S. multivorans*.

Isotope Fractionation during cDCE Dechlorination. The reductive dechlorination of cDCE resulted in larger fractionation effects than dechlorination of TCE (Figure 2). With 90% degradation, cDCE was fractionated by 51.3 and 41.8‰ by *Dehalococcoide* sp. strains 195 and BAV1, respectively. Around twice as large an effect was observed in the *Dehalococcoides*-containing enrichment ANAS with a fractionation of 90.2‰ over a similar 90% degradation. The Rayleigh equation again fit the data well ($r^2 > 0.97$) with ANAS exhibiting the most negative enrichment factor at -29.7 ± 1.6‰ and strain BAV1 the least at -16.9 ± 1.4‰ (Figure 2 and Table 1).

Isotope Fractionation during 1,1-DCE and tDCE Dechlorination. 1,1-DCE was dechlorinated by *Dehalococcoides* sp. strains 195 and BAV1 and the ANAS enrichment to VC. The two *Dehalococcoides* isolates fractionated 1,1-DCE to a much smaller extent than the other chloroethenes. Over 90% degradation, the change in isotope composition of the residual 1,1-DCE from the initial value was 13.8 and 17.6‰ for strains 195 and BAV1, respectively (Figure 3). In contrast, a much larger 1,1-DCE fractionation was observed in ANAS that generated a fractionation of 56.1‰ over 90% degradation (Figure 3). The fit of the Rayleigh equation to the data ($r^2 >$

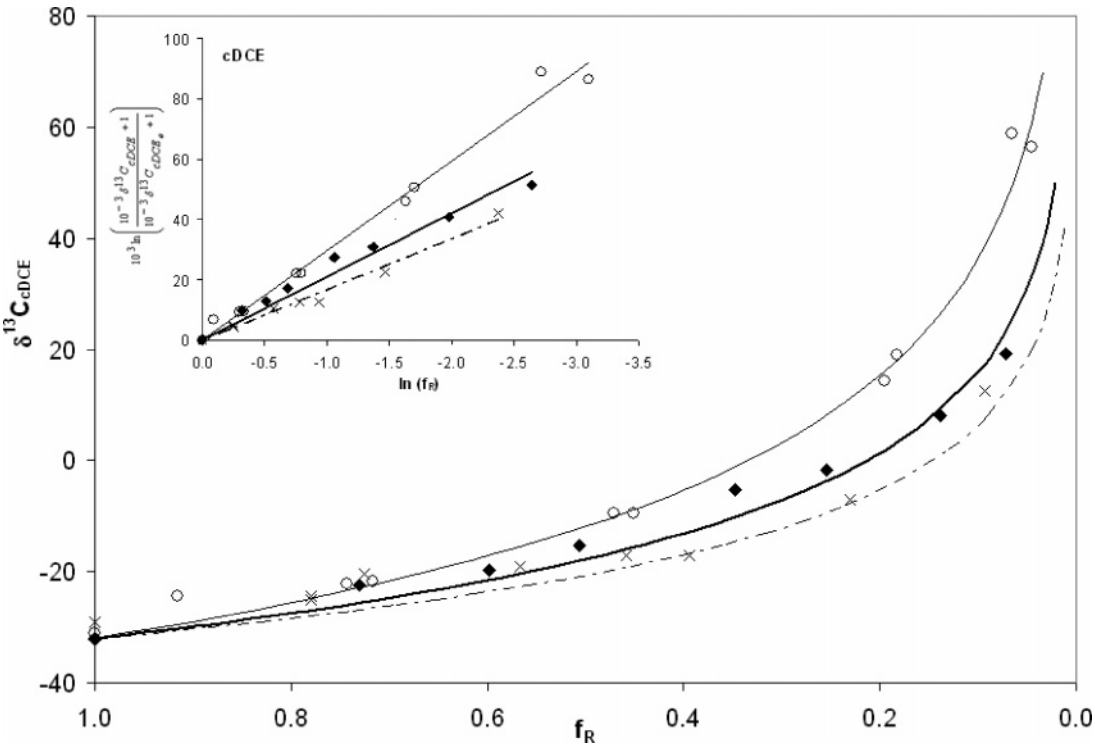

FIGURE 2. Stable carbon isotope fractionation of cDCE by different dechlorinating cultures. Symbols represent measurements from duplicate experimental bottles, and lines are fitted to the Rayleigh model using the calculated ϵ. The inserted graph shows the Rayleigh equation linear regression plot. (◆) and (—) denote strain 195; (×) and (– · – ·) strain BAV1; and (○) and (–) ANAS.

TABLE 1. Compound-Specific Stable Carbon Isotope Rayleigh Enrichment Factors (ϵ) for Reductive Dechlorination of Chloroethenes by Dechlorinating Organisms and Abiotic Reactions Catalyzed by Cyanocobalamin

	TCE			cDCE			1,1-DCE			tDCE			VC		
cultures	ϵ	95% CI	r^2	ϵ	95% CI	r^2	ϵ	95% CI	r^2	ϵ	95% CI	r^2	ϵ	95% CI	r^2
strain 195[f]	−9.6	±0.4	0.99	−21.1	±1.8	0.97	−5.8	±0.5	0.97		n/a[a]			n/a	
strain BAV1[f]		n/a		−16.9	±1.4	0.98	−8.4	±0.3	0.99	−21.4	±0.9	0.99	−24.0	±2.0	0.97
S. multivorans[f]	−16.4	±1.5	0.97		n/a			n/a			n/a			n/a	
D. restrictus strain PER-K23[f]	−3.3	±0.3	0.98		n/a			n/a			n/a			n/a	
ANAS[f]	−16.0	±0.6	0.99	−29.7	±1.6	0.99	−23.9	±1.2	0.98	−28.3	±1.4	0.99	−22.7	±0.8	0.99
KB-1[b]	−2.5	n/a	0.91	−14.1	n/a	0.92							−21.5	n/a	0.99
	−6.6	n/a	1.0	−16.1	n/a	0.99		n/a			n/a		−26.6	n/a	0.96
	−13.8	±0.7	0.98	−20.4	±1.2	0.94							−22.4	±1.8	0.91
Pinellas enrichment[c]	−7.1	n/a	0.98		n/a			n/a			n/a			n/a	
Louisiana microcosm[d]		n/a		−19.9	±1.5	0.98	−7.3	±0.4	0.99	−30.3	±1.9	0.98	−31.1	±0.4	1.0
abiotic with cyanocobalamin[e]	−17.2		0.99												
	−16.6	n/a	0.99		n/a			n/a			n/a			n/a	

[a] n/a: not available because the chloroethene is not a reported metabolic electron acceptor or it was not reported in the referenced study. [b] References *11* and *38*. [c] Reference *37*. [d] Reference *34*. [e] Reference *52*. [f] This study.

0.97) gave enrichment factors for strains 195 and BAV1 of −5.8 ± 0.5 and −8.4 ± 0.3‰, respectively (Figure 3 and Table 1), and of −23.9 ± 1.2‰ for the ANAS enrichment.

Over the reductive dechlorination of 90% of tDCE to VC by strain BAV1 and ANAS, the cultures fractionated the residual tDCE by 54.6 and 61.0‰, respectively (Figure 4). Consistent with the other chloroethenes, the Rayleigh equation fit the data well ($r^2 > 0.97$) with enrichment factors for strain BAV1 and ANAS of −21.4 ± 0.9 and −28.3 ± 1.4‰, respectively (Figure 4 and Table 1).

Isotope Fractionation during VC Dechlorination. Isotopic fractionation caused by the reductive dechlorination from VC to ethene was measured for *Dehalococcoides* sp. strain BAV1 and the ANAS enrichment (Figure 5). Over 85% degradation of VC, the residual VC was fractionated by 44.9 and 47.8‰ for BAV1 and ANAS, respectively. The Rayleigh equation fit the data well ($r^2 > 0.97$) and generated enrichment factors of −22.7 ± 0.8 to −24.0 ± 2.0‰ (Figure 5 and Table 1).

Isotope Ratio in Abiotic Controls. Carbon isotope ratios remained constant and comparable to those of the pure phase chloroethene in all of the abiotic control bottles that were incubated simultaneously with each culture (data not shown), confirming that the biological reactions were responsible for the isotope shifts.

Discussion

Significant carbon isotope fractionation has been observed for reductive dechlorination of chloroethenes by isolates reducing PCE to TCE (*13*), a variety of enrichment cultures

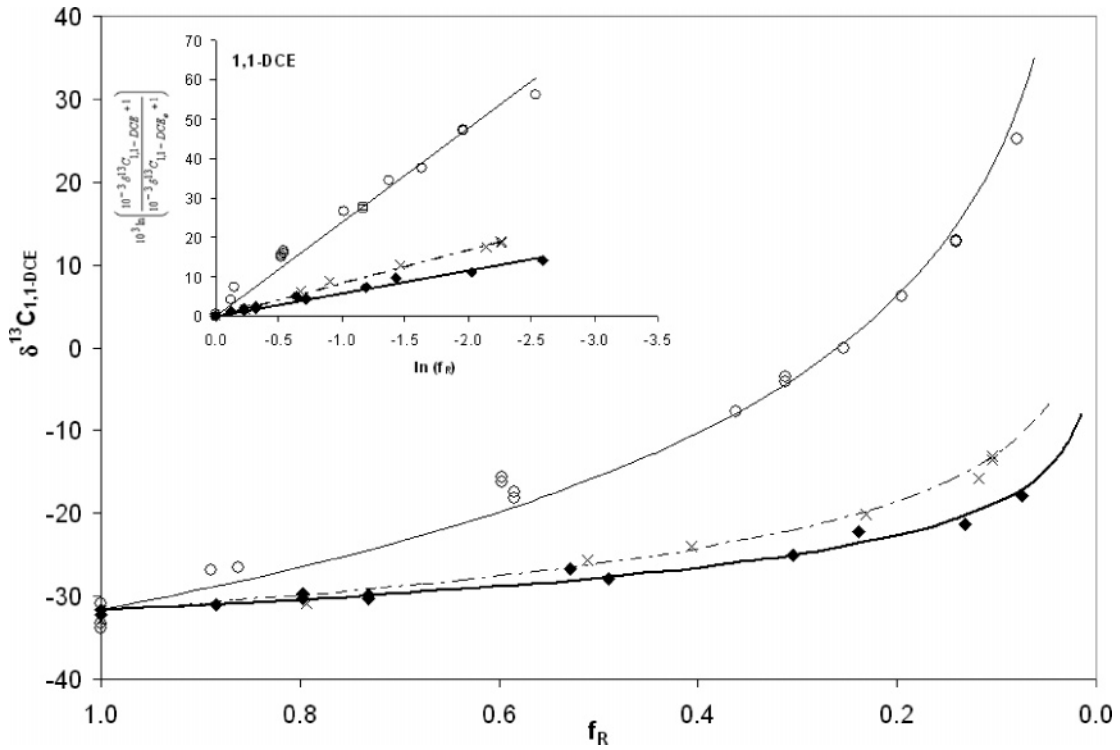

FIGURE 3. Stable carbon isotope fractionation of 1,1-DCE by different dechlorinating cultures. Symbols represent measurements from duplicate experimental bottles, and lines are fitted to the Rayleigh model using the calculated ϵ. The inserted graph shows the Rayleigh equation linear regression plot. (◆) and (—) denote strain 195; (×) and (— · — ·) strain BAV1; and (○) and (—) ANAS.

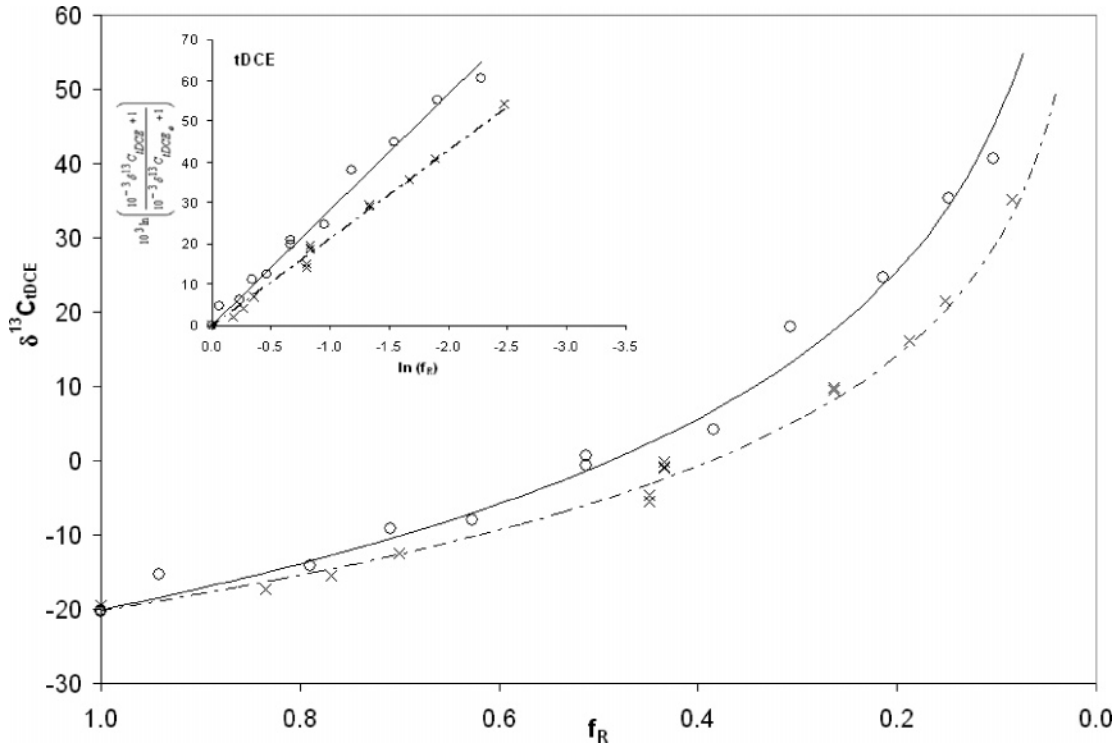

FIGURE 4. Stable carbon isotope fractionation of tDCE by different dechlorinating cultures. Symbols represent measurements from duplicate experimental bottles, and lines are fitted to the Rayleigh model using the calculated ϵ. The inserted graph shows the Rayleigh equation linear regression plot. (×) and (— · — ·) denote strain BAV1 and (○) and (—) ANAS.

(*11*, *34*, *37*, *38*), and in field studies (*12*, *14*−*16*). This study reports the first measurement of carbon isotope fractionation by *Dehalococcoides* isolates and of TCE transformation by *S. multivorans* and *D. restrictus* strain PER-K23. Across phylogenetically distinct organisms and within the *Dehalococcoides* genus dechlorinating different chloroethenes, the extent of fractionation was observed to vary widely. However, consistently, the change in isotope ratios for each culture

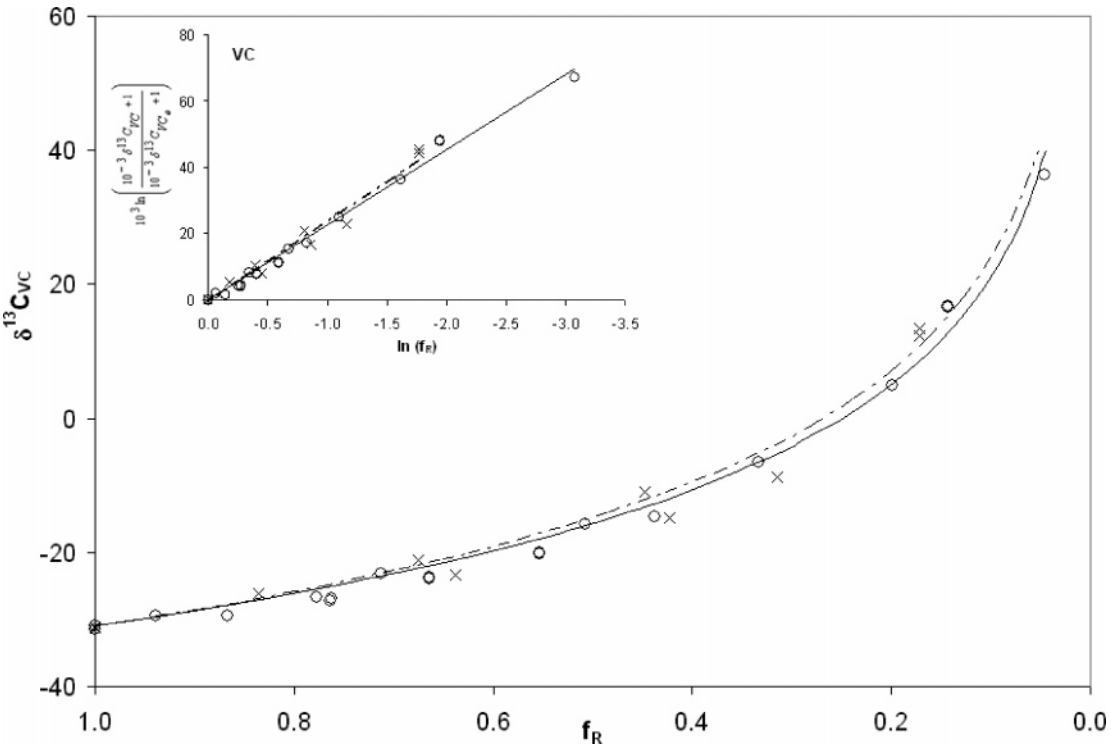

FIGURE 5. Stable carbon isotope fractionation of VC by different dechlorinating cultures. Symbols represent measurements from duplicate experimental bottles, and lines are fitted to the Rayleigh model using the calculated ϵ. The inserted graph shows the Rayleigh equation linear regression plot. (×) and (— · — ·) denote strain BAV1 and (○) and (—) ANAS.

followed the Rayleigh model ($r^2 > 0.97$), giving a constant enrichment factor.

Carbon isotope fractionation of TCE by the three phylogenetically and metabolically diverse isolates studied here offers an interesting comparison and insight into factors that affect biological fractionation. *D. restrictus* strain PER-K23 (*45*), from the Firmicutes phylum, and *S. multivorans* (*43*, *44*), from the ϵ-subclass of the Proteobacteria phylum, are two dechlorinating organisms that use PCE and TCE as their metabolic electron acceptors and cannot dechlorinate beyond DCE. In contrast, *D. ethenogenes* 195, from the Chloroflexi phylum, uses PCE, TCE, cDCE, and 1,1-DCE as metabolic electron acceptors and co-metabolically reduces VC and tDCE to ethene (*19*, *39*, *51*). As shown in Table 1, a range of enrichment factors varying by as much as 5-fold was measured across these TCE dechlorinating organisms. Interestingly, *S. multivorans* exhibited an enrichment factor that was closer to the enrichment culture ANAS than to the *D. restrictus* strain PER-K23 which is more metabolically similar in electron acceptor usage.

Despite the phylogenetic and metabolic differences, the RDases of the TCE-reducing organisms studied here are corrinoid-dependent and require iron−sulfur groups as cofactors (*18*). Abiotic carbon isotope fractionation of TCE by cyanocobalamin (a form of vitamin B-12) has been reported to exhibit enrichment factors between −16.2 to −17.2‰ (Table 1) (*52*). This range is closest to that of *S. multivorans* and ANAS but is more negative than those exhibited by *D. ethenogenes* 195 and *D. restrictus* strain PER-K23 (Table 1). This result was especially interesting since the corrinoid of the PCE-RDase (that also catalyzes TCE reduction) in *D. restrictus* strain PER-K23 is identical in structure to the commercially available cyanocobalamin used in the abiotic study (*30*). Differences in reaction rates between purified TCE-RDase from *D. ethenogenes* 195 and abiotic cyanocobalamin demonstrate that there are mechanistic differences between the activities of free cyanocobalamin and the native RDase in TCE reduction (*28*).

The similarity in enrichment factors for *S. multivorans* and the abiotic cyanocobalamin reaction was also interesting because the corrinoid cofactor of *S. multivorans* is a newly described type of natural corrinoid termed nor-pseudo B_{12} (*53*), and the chloroethene conversion by that organism is mediated by the native protein rather than by the corrinoid cofactor (*27*). The variations in fractionation factors observed for abiotic and biotic TCE reduction suggest that other factors such as cellular transport or enzyme affinity may influence biological isotope fractionation. In summary, although corrinoid is a cofactor of the identified functional RDases of all three isolates studied here, there was no observable trend in the biological and abiotic cyanocobalamin-catalyzed isotope fractionation.

Kinetic processes such as transport of substrate across cell membranes and enzyme−substrate binding can also influence isotopic fractionation. In fact, a systematic comparison of carbon isotope fractionation by growing cells, crude extracts, and purified enzyme of *S. multivorans* reducing PCE to TCE demonstrated that a decrease in cell integrity correlated with an increase in isotope fractionation (*13*). While steps prior to the conformational change of a chemical bond may not themselves result in kinetic isotope fractionation, their effects on the kinetics of the reaction can affect the observed isotope fractionation (*13*). The large range of TCE enrichment factors observed in this study coupled with the reported similarities of the RDases suggest that these biological fractionations are governed by a combination of the structure of the native enzyme and its cofactor, transport, and enzyme−substrate binding prior to the carbon−chlorine bond-breaking step. Given that a number of factors could potentially control reductive dechlorination fractionation effects, generalizations or predictions of enrichment factors

of a given system without specific experimental measurements would be problematic.

Differences in enrichment factors within the same strain reducing different chloroethenes were also observed in both *Dehalococcoides* strains (Table 1) and *S. multivorans*. These differences could be a function of different enzymes catalyzing the reactions and/or the structure of the chloroethenes. In strain 195, the TCE-RDase has been reported to catalyze TCE, cDCE, and 1,1-DCE reduction (*28*, *29*). However, the different reduction rates observed for these compounds by the purified TCE-RDase suggests that the enzyme has different substrate-enzyme binding affinities (*29*). The different chemical structures could also lead to preferential transport across membranes. Furthermore, 17 intact putative RDase genes have been identified in the genome of strain 195 (*19*), and the participation of one or more of the putative RDases in different chloroethene reductions could lead to different fractionation patterns. Similarly, in strain BAV1, the three DCE isomers and VC each had different enrichment factors (Table 1). Genetic analysis has implicated the *bvcA* gene as the functionally important RDase in this strain which also contains six other putative RDase genes (*23*). cDCE and 1,1-DCE are the two common metabolic electron acceptors between strains 195 and BAV1, and differences in enrichment factors are observed (Table 1). This is not surprising since the *tceA* gene that is functional in strain 195 is absent in strain BAV1 and the *bvcA* gene in strain BAV1 is not found in strain 195 (*19*, *23*, *25*). The two strains also share no identical putative RDase genes (*25*). Yet, it is interesting to note that even though there are differences in enrichment factors between 1,1-DCE reduction between the two strains, the values for this compound are consistently less negative than those of other chloroethenes (Table 1).

For growing cells of *S. multivorans*, the reported enrichment factor for PCE reduction was -0.42 ± 0.08‰ (*13*), a value that is more than an order of magnitude less negative than the enrichment factor measured for TCE in this study. Since both PCE and TCE activities in *S. multivorans* are catalyzed by the same RDase (*27*, *30*), the widely varying enrichment factors reinforce the concept that a single enzyme may generate significantly different fractionation effects with different substrates.

The enrichment factors measured for the TCE enrichment culture ANAS varied significantly from those observed for the isolates. This is especially noticeable for 1,1-DCE reduction where ANAS exhibited an enrichment factor 3−4-fold more negative than the two isolates of *Dehalococcoides* (Table 1). Comparison of dechlorination enrichment factors across different enrichment cultures listed in Table 1 suggests that microbial communities possess unique enrichment factors that do not seem to follow observable trends. This could be a result of the growth conditions and the different compositions of the communities including the presence of different strains of *Dehalococcoides* and other dechlorinating organisms. The *Dehalococcoides*-containing enrichment KB-1 contains two RDase genes that are similar to *bvcA* and *vcrA* but the *tceA* gene is noticeably absent (*26*). Conversely, ANAS studied here contains the *tceA* and *vcrA* genes of *Dehalococcoides*, but the *bvcA* gene is not detectable (*42*). The genes present in the Pinellas enrichment (*37*) and Louisiana microcosms (*34*) have not yet been characterized.

Compound-specific stable isotope analysis has become a valuable tool for assessing in situ bioremediation in subsurface environments where many physical and biological processes can occur simultaneously. Rayleigh enrichment factors are useful for quantitatively interpreting isotopic data and for applying predictive isotopic analysis (*33*−*36*). However, due to the wide variation in Rayleigh enrichment factors measured for both bacterial isolates and enriched communities, it is important to estimate factors that are representative of the actual organisms catalyzing the reaction of interest in a contaminated field site where the microbial composition and growth conditions can influence the enrichment factors. As pointed out by Nijenhuis et al. (*13*), applying an incorrect enrichment factor can lead to significant errors in estimating the degradation rates. As shown in this study, dechlorinating cultures can exhibit a range of enrichment factors at each dechlorination step, and strains within the same genus or species can generate significantly different enrichment factors. Further, isotope fractionation generated by a microbial community can be quite different from that generated by isolates, suggesting that general categorization of enrichment factors without specific measurements can introduce inaccuracy and that caution should be exercised in selecting appropriate values for quantitative analysis to predict the extent of dechlorination.

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