Stable Carbon Isotope Fractionation of 1,2-Dichloropropane during Dichloroelimination by Dehalococcoides Populations

Kelly E. Fletcher, Frank E. Löfﬂer, Hans-Hermann Richnow, and Ivonne Nijenhuis

School of Civil and Environmental Engineering and School of Biology, Georgia Institute of Technology, 311 Ferst Drive, Atlanta, Georgia 30332, and Department of Isotope Biogeochemistry, UFZ-Centre for Environmental Research, Leipzig-Halle GmbH, Permoserstr. 15, D-04318 Leipzig, Germany

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The isotope fractionation of 1,2-dichloropropane (1,2-D) during dichloroelimination to propene by Dehalococcoides populations was explored in laboratory experiments in order to provide data for the characterization of the fate of 1,2-D in heterogeneous subsurface systems. Compound specific stable carbon isotope analysis (CSIA) was used to determine the bulk enrichment factors ($\epsilon_{\text{bulk}}$), reactive position specific enrichment factors ($\epsilon_{\text{reactive}}$), and apparent kinetic isotope effect (AKIE) values for 1,2-D dichloroelimination in two distinct Dehalococcoides-containing cultures. The $\epsilon_{\text{bulk}}$ factors calculated in the two cultures were statistically identical, $-10.8 \pm 0.9$ and $-11.3 \pm 0.8$, even though the cultures were derived from geographically distinct locations. AKIE values for 1,2-D dichloroelimination assuming stepwise and concerted reaction mechanisms were approximately 1.033 and 1.017, respectively. These values are within the range of previously reported values for biotic 1,2-dichloroethane and abiotic 1,1,2,2-tetrachloroethane dichloroelimination reactions.

Introduction

1,2-Dichloropropane (1,2-D) has been used extensively as a soil fumigant in agriculture and is generated during the production of propylene oxide and chlorinated solvents, including tetrachloroethene and carbon tetrachloride (1-4). 1,2-D is a potential carcinogen and, therefore, is regulated by the U.S. Environmental Protection Agency at a maximum contaminant level in drinking water of 5 ppb (5). Because 1,2-D is a contaminant of concern at over 100 U.S. Superfund sites (6), tools to assess the fate of 1,2-D in subsurface environments are needed.

Microcosm and field studies have demonstrated that 1,2-D is recalcitrant under aerobic and nitrate-reducing conditions, but degraded under anaerobic conditions (7). BL-DC-8 and BL-DC-9, isolates comprising a novel genus within the Chloroflexi phylum (8), Dehalobacter populations (9), Dehalococcoides populations (10), and Desulfotibacterium dichloroeliminans strain DCA1 (11) can transform 1,2-D to propene under anaerobic conditions. Strains BL-DC-8, BL-DC-9, DCA1, and Dehalococcoides populations convert 1,2-D to propene via a dichloroelimination reaction without the formation of intermediates (3, 8, 11). In subsurface environments, however, physical processes such as sorption, volatilization, and dilution also affect the concentration and fate of 1,2-D.

In general, quantitatively differentiating the effects of biotransformations from physical processes on contaminants in heterogeneous subsurface systems is challenging (12). Compound specific stable carbon isotope analysis (CSIA) is used to quantify the isotope enrichment of compounds undergoing biodegradation and has been applied to assess the in situ biodegradation of chlorinated ethenes (13-18) and chlorinated ethanes (13, 14). To apply CSIA for quantifying in situ contaminant degradation using the Rayleigh equation, the carbon isotope enrichment factor ($\epsilon_{\text{bulk}}$) is used as a parameter (19). The $\epsilon_{\text{bulk}}$ Factor is derived from defined laboratory experiments, where the only sink of the contaminant is, for example, biodegradation. Laboratory-derived $\epsilon_{\text{bulk}}$ factors can also be converted to reactive position specific enrichment factors ($\epsilon_{\text{reactive}}$) and apparent kinetic isotope effect (AKIE) values to obtain information about the biochemical transformation mechanism. Because AKIE values are corrected for the isotope composition of atoms that are not at the reactive site and for the effects of intramolecular competition, AKIE values describe the isotope effect associated specifically with the chemical bond cleavage (20). The semiempirical Streitwieser limits provide reaction-specific maximum values for isotope effects which can be used as a simplified theoretical framework for interpretation of AKIE values (21). While position-specific isotope analysis provides even greater information regarding bond cleavage, CSIA was performed in this study because it is applicable to environmental samples.

In this study, $\epsilon_{\text{bulk}}$ and $\epsilon_{\text{reactive}}$ factors for 1,2-D dichloroelimination were determined in two highly enriched, Dehalococcoides-containing cultures derived from geographically distinct locations (10). This is the first report of isotope enrichment factors for 1,2-D dichloroelimination. Furthermore, using previously reported $\epsilon_{\text{bulk}}$ factors, AKIE values for other dichloroelimination reactions were calculated to serve as a comparison to values for 1,2-D dichloroelimination.

Experimental Section

Cultures and Medium Preparation. Nonmethanogenic RC and KS enrichment cultures were derived from sediments from the Red Cedar River in Michigan and the King Salmon River in Alaska, respectively (10). Duplicate cultures were inoculated (3% vol/vol) into anoxic, bicarbonate buffered (30 mM) mineral salts medium (22) amended with vitamins (23), 1.4 mM Th(III) citrate, 5 mM acetate, and 3-4 mL of gaseous H$_2$. RC and KS cultures were amended with 1.7 and 1.1 mL of 1,2-D dissolved in 0.1 mL methanol, respectively. Cultures were incubated at 25 °C in 160 mL serum bottles containing approximately 100 mL of medium.

Analytical Techniques. To quantify 1,2-D and propene concentrations, headspace or aqueous samples were col-
TABLE 1. Compound Specific Values Used for the Calculation of AKIE Values

<table>
<thead>
<tr>
<th>fractionating Compound</th>
<th>$n$</th>
<th>$x$</th>
<th>$z$</th>
<th>$n$</th>
<th>$x$</th>
<th>$z$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-D</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1,2-DCA</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1,1,2-TCA</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>1,1,2,2-TeCA</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>pentachloroethane</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
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</tr>
<tr>
<td>hexachloroethane</td>
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<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ-HCH</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*No correction for nonreactive positions is required in symmetric molecules and therefore the “$n/x$” term in eq 4 is equal to 1 (27).

FIGURE 1. The carbon isotope composition of 1,2-D (circles) and propene (triangles) in cultures RC (A) and KS (B) during 1,2-D dichloroelimination. Dashed lines indicate the expected isotope composition of the total volatile organics in the system (i.e., 1,2-D and propene) corrected based on losses due to sample removal. Isotope values are reported in δ-notation relative to the Vienna Pee Dee Belemnite (PDB) standard. Data were averaged from duplicate isotope measurements and error bars depict one standard deviation (1σ).

lected and measured using a gas chromatograph (GC) as described previously (24, 25). For analysis of carbon isotope composition, 7 mL aqueous samples were collected and placed in 10 mL vials with 1 mL of 1 M NaOH to inhibit metabolic activity. Sample vials were sealed with Teflon-lined septa and stored at 4 °C. Each sample vial was analyzed a minimum of two times using a GC combustion isotope ratio mass spectrometer, and carbon isotope compositions were quantified as described previously (25), except that sample vials were heated to 60 °C prior to the removal of 0.7 mL headspace samples.

Calculations. Stable carbon isotope $\varepsilon_{\text{bulk}}$ factors for 1,2-D dichloroelimination were calculated according to the Rayleigh model (21, 25)

$$\ln\left(\frac{(1000 + \Delta^{13}C_{\text{n}} + \Delta^{13}C_{\text{d}})}{(1000 + \Delta^{13}C_{\text{n}})}\right) = \left(\varepsilon_{\text{bulk}} / 1000\right)\ln(f) \quad (1)$$

where $\Delta^{13}C_{\text{n}}$ is the carbon isotope composition of 1,2-D at time zero, $\Delta^{13}C_{\text{d}}$ is the change in the carbon isotope composition from time zero to time $t$, and $f$ is the molar fraction of 1,2-D remaining at time $t$. Specifics regarding the calculation of $f$ are provided in the Supporting Information (SI). Carbon isotope $\varepsilon_{\text{reactive}}$ factors, which are corrected for the presence of nonreactive positions, were calculated for both stepwise and concerted reactions according to ref 21

$$\ln\left(\frac{(1000 + \Delta^{13}C_{\text{n}} + (n/x)\Delta^{13}C_{\text{d}})}{(1000 + \Delta^{13}C_{\text{n}})}\right) = \left(\varepsilon_{\text{reactive}} / 1000\right)\ln(f) \quad (2)$$

where $n$ is the number of carbon atoms in the molecule (in the case of 1,2-D, $n = 3$) and $x$ is the number of carbon atoms in the reactive position (in the case of a stepwise reaction, $x = 1$ and in the case of a concerted reaction, $x = 2$, assuming identical KIE values). AKIE values for 1,2-D dichloroelimination were calculated according to ref 21

$$\text{AKIE} = 1/(1 + (z \times \varepsilon_{\text{reactive}} / 1000)) \quad (3)$$

where $z$, the number of indistinguishable reactive sites, is a correction for the effects of intramolecular competition (in the case of 1,2-D, $z = 1$). In order to compare carbon isotope fractionation during various dichloroelimination reactions, AKIE values for other dichloroelimination reactions were approximated from reported $\varepsilon_{\text{bulk}}$ values according to ref 21

$$\text{AKIE} = 1/(1 + z \times (n/x) \times \varepsilon_{\text{bulk}} / 1000) \quad (4)$$

where compound-specific values for $z$, $n$, and $x$, are shown in Table 1.

Results and Discussion

Enrichment of $\delta^{13}C$ in 1,2-D during Microbially Catalyzed Dichloroelimination to Propene. In freshly inoculated RC cultures, dichloroelimination to propene began after a lag period of 3 days. More than 90% of 1,2-D was transformed to propene within 6 days after the lag period. Similarly, in KS cultures, more than 90% of 1,2-D was transformed to propene in 11 days after a lag period of approximately 15 days. The 1,2-D transformation rates were 2.57 ± 0.07 and 1.08 ± 0.12 μmoles per day in RC and in KS cultures, respectively. In both cultures, 1,2-D was significantly enriched in $\delta^{13}C$ during transformation to propene (Figure 1A and B). The initial 1,2-D isotope composition in cultures RC and KS differed because 1,2-D was obtained from different sources (Supelco for culture RC; Riedel-de Haën for culture KS). Isotopically depleted propene was formed in both cultures, verifying isotope fractionation.

In both RC and KS cultures, the total amount of volatile organics in the system (i.e., 1,2-D and propene) became depleted in $\delta^{13}C$ as degradation continued; yielding a poor isotope balance. For example, in KS cultures that had consumed 1,2-D to below the detection limit of 0.5 mg/L, the isotope composition of propene was $-27.9 ± 0.3%$o, which is significantly depleted in $\delta^{13}C$ compared to the initial isotope composition of 1,2-D, which was $-24.3 ± 0.5%$o. The depletion of $\delta^{13}C$ in the cumulative organic volatiles in the cultures was a result of the preferential removal of 1,2-D during isotope composition sample collection. Because Henry’s constants for 1,2-D and propene are 0.12 and 8.54, respectively, approximately 91% (mol/mol) of 1,2-D is in the aqueous phase while only approximately 11% (mol/mol) of propene is in the aqueous phase in these cultures. Only the aqueous phase was sampled for isotope analysis of 1,2-D and propene, and therefore, about 4- to 5-fold more 1,2-D than propene was removed, resulting in the preferential removal of $\delta^{13}C$-enriched substrate and leading to a depletion of $\delta^{13}C$ in the cultures. Correcting the isotope balance for the losses due to sample removal (calculation details are provided in the SI), the expected isotope composition in GS cultures following complete transformation of 1,2-D was calculated to be $-28.3 ± 1.3%o$. This value is statistically identical to the final isotope composition of propene, $-27.9 ± 0.3%o$, demonstrating a closed isotope balance (Figure 1B). The depletion of $\delta^{13}C$ in RC cultures was accounted for in the same manner. The final isotope composition of propene in the RC cultures was lower than the expected isotope composition likely because only 93% of the initial dose of 1,2-D was transformed to propene prior to collection of the final sample (Figure 1A).

Bulk Stable Carbon Isotope Enrichment Factors ($\varepsilon_{\text{bulk}}$). In both cultures, the Rayleigh model described isotope fractionation during transformation of 1,2-D to propene
were statistically identical (Table 2). These findings suggest that carbon isotope ε\textsubscript{bulk} factors for 1,2-D transformation by 

\begin{table}
\centering
\begin{tabular}{llllll}
\hline
fractionating compound & reactant & ε\textsubscript{bulk} & stepwise & concerted & reference \\
\hline
1,2-D & culture RC & $-10.8 \pm 0.9$ & 1.0325 & 1.0164 & this study \\
 & culture KS & $-11.3 \pm 0.8$ & 1.0332 & 1.0167 & this study \\
 & cultures A and B & $-7.3 \pm 0.2$ & 1.0148 & 1.0074 & 13 \\
 & cultures C and D & $-16.7 \pm 0.5$ & 1.0346 & 1.0170 & 13 \\
 & LA microcosm & $-32.1 \pm 1.1$ & 1.0686 & 1.0332 & 29 \\
 & Zn(II) & $-29.7 \pm 1.5$ & 1.0632 & 1.0306 & 30 \\
1,1,2-DCA & Cr(II) & $-18.0 \pm 0.5$ & 1.0374 & 1.0184 & 27 \\
 & Cr(II) & $-12.7 \pm 1.2$ & 1.0261 & 1.0129 & 28 \\
 & Cu-plated iron & $-17.0 \pm 0.6$ & 1.0351 & 1.0173 & 27 \\
 & Fe & $-19.3 \pm 0.7$ & 1.0401 & 1.0196 & 27 \\
pentachloroethane & Cr(II) & $-14.7 \pm 0.6$ & 1.0303 & 1.0149 & 28 \\
hexachloroethane & Cr(II) & $-10.4 \pm 0.5$ & 1.0212 & 1.0105 & 28 \\
γ-HCH & D. gigas & $-4.0 \pm 0.2$ & 1.0246 & 1.0121 & 26 \\
 & D. multivorans & $-3.4 \pm 0.2$ & 1.0208 & 1.0103 & 26 \\
\hline
\end{tabular}
\caption{Stable Carbon Isotope ε\textsubscript{bulk} and Calculated AKIE Values Assuming Both Stepwise and Concerted Dichloroelimination Reactions}
\end{table}

AKIE values were calculated assuming both stepwise and concerted scenarios.

Assuming that the 1,2-D dichloroelimination reaction is stepwise, involving the cleavage of one C–Cl bond in the transition state, ε\textsubscript{reactive} factors are −31.5 ± 2.7 and −32.1 ± 2.5% and AKIE values were 1.0325 ± 0.0029 and 1.0332 ± 0.0027 for cultures RC and KS, respectively. Assuming that the reaction is concerted, involving the simultaneous cleavage of both C–Cl bonds, AKIE values were 1.0164 ± 0.0014 and 1.0167 ± 0.0013 for cultures RC and KS, respectively (Table 2). The Streitwieser semiclassical limit for the KIE of the cleavage of a C–Cl bond is 1.057, but measured AKIE values are generally below this value due to the effects of slow, nonfractionating steps preceding bond cleavage, (e.g., binding of the substrate to the enzyme). Furthermore, the Streitwieser limits assume that bonds are completely broken in the transition state of a reaction which is not always the case (21), but these limits do allow for a broad mechanistic interpretation. Therefore, if AKIE values calculated assuming a stepwise reaction are greater than the Streitwieser limit, the reaction likely proceeds via a concerted mechanism according to a broad classification. In the case of dichloroelimination of 1,2-D, however, the reaction mechanism cannot be absolutely classified because AKIE values calculated assuming both stepwise and concerted reaction mechanisms are significantly below 1.057.

The AKIE values for 1,2-D dichloroelimination were compared to values for dichloroelimination of 1,2-dichloroethane (1,2- DCA), 1,1,2-trichloroethane (1,1,2-TCA), 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA), pentachloroethane, hexachloroethane, and gamma-hexachlorocyclohexane (γ-HCH) (13, 26–30), which were calculated assuming that the effects of secondary KIEs are negligible. The dichloroelimination of 1,2-DCA by two distinct mixed cultures (13), of 1,1,2,2-TeCA by Cu-plated iron (27), and of pentachloroethane by Cr(II) (28) demonstrate AKIE values highly similar to those calculated for the Dehalococcoides-catalyzed 1,2-D dichloroelimination reaction (Table 2). Interestingly, Dehalococcoides populations also catalyzed the dichloroelimination of 1,2-DCA in mixed culture D, (also known as KB-1 (31)) and most likely, at least partially, in culture C (Table 2 (32)), which

(FIGURE 2) Although the enrichment cultures were obtained from geographically distinct locations, harbor unique microbial populations (10), and demonstrate different 1,2-D dichloroelimination rates, the ε\textsubscript{bulk} factors calculated for RC and KS cultures, −10.8 ± 0.9 and −11.3 ± 0.8%, respectively, were statistically identical (Table 2). These findings suggest that carbon isotope ε\textsubscript{bulk} factors for 1,2-D transformation by Dehalococcoides populations are consistent and therefore applicable to demonstrate in situ natural attenuation of 1,2-D and to assess the extent of 1,2-D biodegradation, although it would be recommendable to corroborate these findings with further studies.

**Apparent Kinetic Isotope Effect (AKIE) Values.** AKIE values were calculated to characterize the isotope effect of the cleavage of the chemical bond at the reactive position of the 1,2-D molecule. While two C–Cl bonds are cleaved during the dichloroelimination reaction, it is unknown if the reaction proceeds via a stepwise mode (i.e., one C–Cl bond is broken in the transition state and the rate-limiting step involves only one carbon atom), or via a concerted mode (i.e., two C–Cl bonds are broken simultaneously and the rate-limiting step involves two carbon atoms). Therefore,
both demonstrated similar AKIE values to those observed for 1,2-D dichloroelimination ([13]; personal communication from E. Edwards).

However, reported AKIE values for dichloroelimination reactions vary widely. As shown in Table 2, AKIE values calculated assuming a concerted reaction mechanism varied from 1.0020 ± 0.0002 for dichloroelimination of 1,1,2-TCA to 1.0332 ± 0.0011 for dichloroelimination of 1,2-DCA, in both cases, in microcosms constructed from Louisiana soil and groundwater (29). Similarly, calculated AKIE values assuming stepwise reactions ranged from 1.0040 ± 0.0004 to 1.0686 ± 0.0022, again, for the dichloroelimination of 1,1,2-TCA and 1,2-DCA, respectively, in microcosms constructed from Louisiana soil and groundwater (29). Interestingly, in this case, the AKIE value for the dichloroelimination of 1,2-DCA assuming a stepwise reaction is greater than 1.057, the Streitwieser semiclassical limit for the cleavage of a C–Cl bond, indicating that the dichloroelimination of 1,2-DCA in the Louisiana microcosm likely occurs via a concerted reaction mechanism (21). However, carbon isotope fractionation during dichloroelimination of 1,2-DCA has been measured in a number of cultures, and calculated AKIE values do not consistently eliminate stepwise reaction mechanisms ([13]. In fact, Hirschorn et al., (2007) ([13] reported that calculated AKIE values vary significantly, from 1.0148 ± 0.0004 to 1.0346 ± 0.0010 assuming a stepwise reaction and from 1.0074 ± 0.0002 and 1.0170 ± 0.0005 assuming a concerted reaction, even among enrichment cultures obtained from the same location and amended with the same electron donor (Table 2). In this case, because enrichment cultures were maintained separately for several years, differences in carbon isotope fractionation may be due to the presence of different microbial populations. Interestingly, microbial lindane dichloroelimination, thought to function via a stepwise reaction, had similar AKIE values (Table 2) ([26]. However, variation in carbon isotope enrichment also occurs in abiotic systems performing the same reaction. For example, Eslner et al., (2009) ([27] and Hofstetter et al., (2007) ([28] both monitored isotope fractionation of 11,1,2,2-TeCA during dichloroelimination by Cr(II), but AKIE values were inconsistent, 1.0374 ± 0.0012 and 1.0261 ± 0.0012, respectively, assuming stepwise reactions and 1.0184 ± 0.0006 and 1.0129 ± 0.0012, respectively, assuming concerted reactions.

These results demonstrate that AKIE values may vary significantly based on the microbial community or experimental procedures employed and that AKIE values are certainly not consistent for dichloroelimination reactions in general. Therefore, it is particularly remarkable that the 1 multifer and consequently, AKIE values calculated for dichloroelimination of 1,2-D are statistically identical in two distinct enrichment cultures. The consistency of the isotope effects between these cultures indicates that CSIA may be a promising approach to verify and quantify 1,2-D dichloroelimination in subsurface environments.

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Supporting Information Available
Additional information regarding the calculations used to determine the fraction of 1,2-D remaining and to correct the isotope balance for the removal of samples. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(20) Hirschorn, S. K.; Dinglasan-Panlilio, M. J.; Edwards, E. A.; Lacrampe-Couloume, G.; Lollar, B. S. Isotope analysis as a natural reaction probe to determine mechanisms of biodeg-


(22) Adrian, L.; Manz, W.; Szewzyk, U.; Gorisch, H. Physiological characterization of a bacterial consortium reductively dechlorinating 1,2,3- and 1,2,4-trichlorobenzene. *Appl. Environ. Microbiol.* 1998, 64 (2), 496–503.


