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Distinct Dual C–Cl Isotope Fractionation Patterns during Anaerobic Biodegradation of 1,2-Dichloroethane: Potential To Characterize Microbial Degradation in the Field

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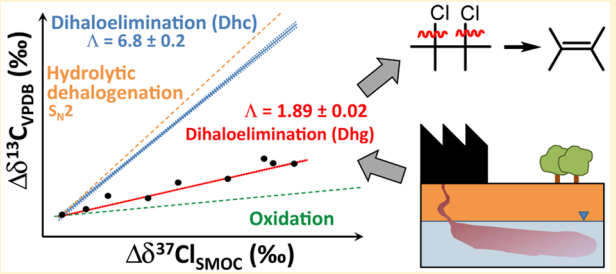
Supporting Information

ABSTRACT: This study investigates, for the first time, dual C–Cl isotope fractionation during anaerobic biodegradation of 1,2-dichloroethane (1,2-DCA) via dihaloelimination by *Dehalococcoides* and *Dehalogenimonas*-containing enrichment cultures. Isotopic fractionation of 1,2-DCA (ε^{C}_{bulk} and ε^{Cl}_{bulk}) for *Dehalococcoides* (−33.0 ± 0.4‰ and −5.1 ± 0.1‰) and *Dehalogenimonas*-containing microcosms (−23 ± 2‰ and −12.0 ± 0.8‰) resulted in distinctly different dual element C–Cl isotope correlations ($\Lambda = \Delta\delta^{13}C/\Delta\delta^{37}Cl \approx \varepsilon^{C}_{bulk}/\varepsilon^{Cl}_{bulk}$), 6.8 ± 0.2 and 1.89 ± 0.02, respectively. Determined isotope effects and detected products suggest that the difference on the obtained Λ values for biodihaloelimination could be associated with a different mode of *concerted* bond cleavage rather than two different reaction pathways (i.e., *stepwise* vs *concerted*). Λ values of 1,2-DCA were, for the first time, determined in two field sites under reducing conditions (2.1 ± 0.1 and 2.2 ± 2.9). They were similar to the one obtained for the *Dehalogenimonas*-containing microcosms (1.89 ± 0.02) and very different from those reported for aerobic degradation pathways in a previous laboratory study (7.6 ± 0.1 and 0.78 ± 0.03). Thus, this study illustrates the potential of a dual isotope analysis to differentiate between aerobic and anaerobic biodegradation pathways of 1,2-DCA in the field and suggests that this approach might also be used to characterize dihaloelimination of 1,2-DCA by different bacteria, which needs to be confirmed in future studies.



INTRODUCTION

Chlorinated ethanes like 1,2-dichloroethane (1,2-DCA) are groundwater contaminants found at many contaminated sites due to improper disposal practices and accidental releases. For instance, 1,2-DCA was detected in 36% of 1585 National Priorities List sites identified by the U.S. Environmental Protection Agency (USEPA), which pose a threat to human and ecosystem health due to its high toxicity.[1] 1,2-DCA is widely used as an intermediate in the chemical industry and as a solvent for many applications. In addition, 1,2-DCA was added to leaded gasoline as a lead scavenger.[1] Falta et al.[2] showed the presence of 1,2-DCA at sites contaminated with leaded gasoline and concluded that knowledge of the occurrence and degradation of 1,2-DCA at such sites is necessary for their better management. A number of laboratory microbiological

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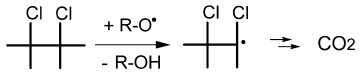
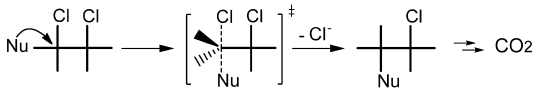
studies reported 1,2-DCA biodegradation under oxic[3−5] and anoxic[6−10] conditions. The high susceptibility of 1,2-DCA to being transformed via distinct biodegradation pathways (Scheme 1), under both oxic and anoxic conditions, complicates the assessment of its fate in the environment, which is relevant information to evaluate the natural attenuation of 1,2-DCA at contaminated sites.

Scheme 1. Aerobic and Anaerobic Biodegradation Pathways of 1,2-DCA in Aqueous Systems[a]

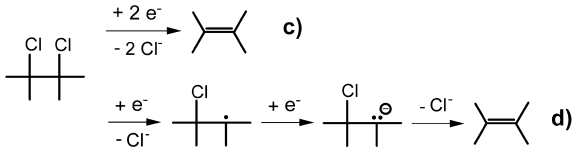
a. Oxidation *(Aerobic)*



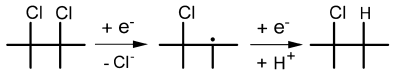
b. Hydrolytic dehalogenation via SN2 *(Aerobic*)*



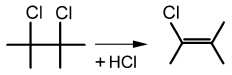
c,d. Dihaloelimination *(Anaerobic)*: **c) concerted, d) stepwise**



e. Hydrogenolysis *(Anaerobic)*



f. Dehydrohalogenation *(Anaerobic)*



[a]* A previous study suggested that hydrolytic dehalogenation also occurs under nitrate-reducing conditions.[39] For the *stepwise* dihaloelimination pathway (d), the carbanion intermediate could also be directly formed by a nucleophilic attack at the halogen atom[51].

Under oxic conditions, 1,2-DCA can be microbially degraded by oxidation[3] (Scheme 1a) and hydrolytic dehalogenation[4,5] (Scheme 1b). Under anoxic conditions, it is usually biodegraded by dihaloelimination[6,7] (either *concerted* or *stepwise* β-elimination, Scheme 1c, d) or hydrogenolysis[10] (Scheme 1e). Initial products of both hydrolytic dehalogenation and aerobic oxidation reactions are further degraded to innocuous end products (Scheme 1a, b). In contrast, chloroethane (CA) can accumulate during biotic reductive dechlorination of 1,2-DCA via hydrogenolysis (Scheme 1e).[10−12] Furthermore, several studies showed transient accumulation of vinyl chloride (VC, Scheme 1f), at concentrations typically much lower than that of ethene, during dihaloelimination of 1,2-DCA by pure *Dehalococcoides* strains[13,14] and a *Dehalococcoides*-containing enrichment culture.[9] Both CA and VC are also groundwater contaminants considered as priority pollutants by the USEPA.[15] Elucidation of active biodegradation pathways for 1,2-DCA under anoxic conditions is thus necessary to predict the potential for accumulation of toxic chlorinated daughter products in the field. At contaminated sites, anoxic conditions are prevalent in groundwater due to depletion of oxygen during degradation of readily oxidizable organic contaminants such as petroleum hydrocarbons, which are often detected at field sites impacted by 1,2-DCA due to its use as a lead-scavenger. Identifying degradation pathways from daughter products analysis is further complicated at sites impacted by mixtures of chlorinated compounds because products can be formed from different precursors. For instance, CA (Scheme 1e) and ethene (Scheme 1c, d) can also be formed from 1,1,1-trichloroethane (1,1,1-TCA)[16] and trichloroethene (TCE),[13] respectively, by reductive dechlorination. Therefore, alternative approaches to detect and identify 1,2-DCA transformation pathways in the environment are warranted.

Single element isotope fractionation analysis is a well-established tool to evaluate the extent of contaminant degradation in the field.[17,18] However, while isotope fractionation of one element alone may also be used to get insight into degradation pathways in laboratory experiments,[19,20] such analysis is not possible under field conditions. The reason is that changes in substrate concentrations at contaminated sites are also related to processes other than transformation, such as sorption or hydrodynamic dispersion. This prevents accurate calculation of isotopic fractionation values (ε_{bulk}) and, hence, precludes mechanistic information based on isotope effects of one element alone. In addition, for carbon, a wide range of ε^{C}_{bulk} values was observed during dihaloelimination of 1,2-DCA in previous laboratory studies, from $-7.3 \pm 0.2‰$ to $-32 \pm 1‰$.[14,21,22] Such variability can reflect the occurrence of different reaction pathways (i.e., *stepwise* versus *concerted* dihaloelimination, Scheme 1c, d) but also the effect of rate-limiting (non or slightly isotope-fractionating) steps preceding the bond cleavage such as contaminant mass transfer,[23] which hampers unambiguous classification of the reaction mechanism.

The situation is different if isotope analysis is conducted on two or more elements (e.g., C and Cl). Combined shifts in isotope ratios of both elements (i.e., $\Delta\delta^{13}C$ vs $\Delta\delta^{37}Cl$) generally exhibit a linear relationship with a slope ($\Lambda = \Delta\delta^{13}C/\Delta\delta^{37}Cl \approx \varepsilon^{C}_{bulk}/\varepsilon^{Cl}_{bulk}$) reflecting the extent of C and Cl isotope effects, which are controlled by chemical bond breakage or formation. Thus, different Λ values may be expected for distinct transformation mechanisms.[24] In contrast to single element isotope fractionation analysis, the proportion of changes in isotope ratios of both elements *relative* to each other ($\Delta\delta^{13}C/\Delta\delta^{37}Cl$) is largely unaffected by nondegradative processes such as contaminant transport.[25−27] Measurements of isotope fractionation of two or more elements are, therefore, crucial to investigate contaminant degradation pathways in the field, which has not been applied to biodegradation field studies of chlorinated ethanes yet.

In a previous laboratory study, Λ values determined during *aerobic* biodegradation of 1,2-DCA by different microbial strains were found to be dependent on the enzymatic mechanism of degradation.[28] Transformation of 1,2-DCA by *Xanthobacter autotrophicus* GJ10[5] and *Ancylobacter aquaticus* AD20[4] (hydrolytic dehalogenation via S_N2-reaction, Scheme 1b) exhibited a Λ value of 7.7 ± 0.2, while transformation by *Pseudomonas* sp. strain DCA1[3] (via aerobic oxidation, Scheme 1a) was associated with a much smaller value of 0.78 ± 0.03, delineating the potential of the dual isotope approach to identify 1,2-DCA degradation pathways in the field.[28] Following this approach, dual isotope patterns observed at contaminated sites can be compared to the laboratory-derived Λ values in order to identify degradation pathways. In contrast to this data on *aerobic* biodegradation, Λ values have not yet been reported for *anaerobic* biodegradation of 1,2-DCA by organohalide-respiring bacteria such as *Dehalococcoides* or *Dehalogenimonas* strains.

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In this study, carbon and chlorine isotopic fractionation (ε^{C}_{bulk} and ε^{Cl}_{bulk}) for microbial dihaloelimination of 1,2-DCA were determined using two laboratory cultures enriched in *Dehalococcoides* and *Dehalogenimonas* populations, respectively. A dual C−Cl isotope approach was used (i) to characterize the Λ value during dihaloelimination of 1,2-DCA; and (ii) to explore the underlying reaction mechanism. In addition, the dual element isotope patterns obtained in this study were compared to data reported for aerobic biodegradation of 1,2-DCA to determine whether the Λ values are sufficiently different to distinguish between dihaloelimination, hydrolytic dehalogenation and aerobic oxidation reactions. Finally, the dual element isotope patterns determined in our laboratory experiments were compared to data observed in groundwater samples from two field sites impacted by 1,2-DCA.

MATERIALS AND METHODS

Biodegradation Batch Experiments. The chemicals and medium used for preparation of microcosms, incubation conditions and sampling details are described in the Supporting Information (SI). Two enrichment cultures (see cultivation details in SI) with different bacterial populations were used for the laboratory experiments. These enrichment cultures were characterized in previous studies[9,29] to determine organohalide-respiring bacteria capable of contaminant degradation. In addition, further characterization was provided in this study for the *Dehalococcoides*-containing culture (see below). Batch tests with *Dehalococcoides* and *Dehalogenimonas*-containing microcosms (performed at Clemson University (CU), and at the Universitat Autònoma de Barcelona (UAB), Spain, respectively) were prepared in an anoxic chamber and the bottles (120 mL total volume) were sealed with Teflon-faced rubber septa and aluminum crimp caps to maintain anoxic conditions.

Batch Experiments with the Dehalococcoides-Containing Culture. The anaerobic enrichment culture was maintained via organohalide respiration of 1,2-DCA, with a *Dehalococcoides* yield of 4.6×10^7 gene copies per μmol Cl^- released.[9] Illumina sequencing of the original culture and the one used in this study indicate that *Dehalococcoides* are the predominant organohalide-respiring microbes present, with either no detections or much lower levels of *Dehalobacter* and *Desulfitobacterium* (SI Figure S1 and Table S1). This analysis also discarded the presence of *Dehalogenimonas*. Since only *Dehalococcoides* increased in gene copy number as 1,2-DCA was consumed,[9] and since *Dehalococcoides* was the predominant dechlorinator present (SI Table S1), we conclude that *Dehalococcoides* were responsible for biodegradation of 1,2-DCA. As shown in the results discussion, this conclusion is in agreement with (i) the detection of VC (typically produced during degradation of 1,2-DCA by pure *Dehalococcoides* strains)[13,14] and (ii) the determined ε^{C}_{bulk} value in the experiments with the *Dehalococcoides*-containing culture (consistent with those reported in a previous study using two different pure *Dehalococcoides* strains).[14] In addition, the excellent correlations of isotope data in both Rayleigh and dual element isotope plots (see below) suggest that a single bacterial population was involved in 1,2-DCA biodegradation.

A total of 30 serum bottles were prepared by dispensing 75 mL of the enrichment culture. 1,2-DCA was added as a water-saturated solution (225 μL per bottle), resulting in an initial aqueous phase concentration of ~25 mg/L (when taking into account partitioning between the headspace and liquid using Henry's Law). Sodium lactate was added to ensure an excess of electron equivalents for dechlorination (150 μL of a sodium lactate stock solution containing 456.2 g/L of 60% sodium lactate syrup).[9] Five killed controls were prepared by adding phosphoric acid first to the bottles (see SI), followed by the 1,2-DCA.

Batch Experiments with the Dehalogenimonas-Containing Culture. Anoxic microcosms were derived from stable enrichment cultures dechlorinating 1,2-dichloropropane to propene. These enrichment cultures were established with sediments from the Besòs river estuary (Spain) after 23 consecutive transfers (3% v/v).[29] Application of genus-specific primers targeting 16S rRNA gene sequences showed the presence of a *Dehalogenimonas* strain, while no amplification was obtained with *Dehalococcoides*-specific primers.[29] Illumina 16S rRNA gene sequencing of this culture indicated that *Dehalogenimonas* was the only known organohalide-respiring bacteria present in the culture capable of dechlorinating 1,2-DCA.

A total of 16 serum bottles were prepared by dispensing 65 mL of a sterilized anoxic medium described elsewhere,[29] with the exception that in half of the samples pyruvate (5 mM) was replaced by acetate (5 mM) as carbon source. The microcosms were inoculated with 3 mL of the *Dehalogenimonas*-containing enrichment culture and 1,2-DCA was added with a syringe from a stock solution in acetone to give an initial aqueous phase concentration of ~9.5 mg/L. Two types of controls were included in this experiment to account for losses, abiotic transformation, and the transfer of compounds from previous degradation experiments with the inoculum or potential impurities from the stock solution: (a) live controls without 1,2-DCA and (b) abiotic controls containing the growth medium with 1,2-DCA but without inoculum.

Description of Field Sites. Groundwater sampling details, redox parameters and site maps are available in the SI (Figure S2, S3 and Table S2). Chlorine and carbon isotope ratios of 1,2-DCA were determined for two industrial sites, named A and B, located in Italy and the U.S., respectively. Both sites showed reducing conditions in groundwater (see SI). Site A is contaminated with petroleum hydrocarbons and 1,2-DCA is the main chlorinated compound present in the subsurface, which consists of Pleistocene-Holocene alluvial deposits. The unconfined aquifer (thickness between 25 and 30 m) is composed of sandy and sandy-loam layers, intercalated by local levels of silt and silty-clay, and the underlying aquitard (average thickness of 25 m) consists of a clayey silt bed.

Site B was impacted by chlorinated hydrocarbons and 1,2-DCA is the main compound detected in groundwater. From top to bottom, the subsurface consists of a silty clay layer (thickness from 8 to 12 m), intercalated by a silty sand layer (up to 3 m thick), and a clay bed corresponding to the low permeability basis of the aquifer (SI Figure S3). Based on concentration data of 1,2-DCA from previous sampling campaigns, selected samples covering different stages of 1,2-DCA biodegradation were collected for isotope analysis at both sites.

Concentration and Isotopic Analyses. A detailed description of analytical methods and equipment used for the isotopic and concentration analysis is available in SI. The concentrations of 1,2-DCA and daughter products in laboratory experiments were measured by headspace analysis using a gas chromatograph-flame ionization detector (GC-FID) at CU[9] and UAB[29] laboratories (see SI). The concentration of 1,2-

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DCA in the abiotic controls of the experiments with *Dehalococcoides* (25.4 ± 0.4 mg/L, n = 5) and *Dehalogenimonas* (9.3 ± 0.9 mg/L, n = 6) containing microcosms remained at the initial concentration along the experiments, which indicates that compound losses through the caps during incubation were insignificant. First-order curve fitting of concentration vs time data from laboratory experiments was performed according to equations (S3−S5, see SI). Chlorinated hydrocarbons in field samples were analyzed by GC−mass spectrometry (GC−MS).

Chlorine isotope measurements of 1,2-DCA in samples from both laboratory experiments were performed at Isotope Tracer Technologies Inc., Canada. For carbon isotope ratios, samples from *Dehalococcoides* and *Dehalogenimonas*-containing microcosms were analyzed at the Universities of Neuchâtel (UN), Switzerland, and Barcelona (UB), Spain, respectively. Carbon and chlorine isotope ratios of 1,2-DCA in groundwater samples from both sites were measured at the University of Waterloo (UW), Canada. Compound-specific carbon and chlorine isotope analyses were performed by GC-isotope ratio mass spectrometry (GC-IRMS). For analyzing chlorine isotope ratios of 1,2-DCA, the two most abundant fragment ions (m/z 62 and 64) were used, which correspond to isotopologue pairs that differ by one heavy chlorine isotope ($[^{37}Cl^{12}C_2{}^1H_3]^+$ and $[^{35}Cl^{12}C_2{}^1H_3]^+$, respectively). The raw δ^{37}Cl values were calibrated to the standard mean ocean chloride (SMOC) scale using two external laboratory standards of 1,2-DCA.[28] These standards were dissolved in water and measured similarly to the samples interspersed in the same sequence.[30]

Duplicate samples and standards were analyzed. Further details about analysis of the samples and standards, for both C and Cl isotopes, as well as calibration of raw δ^{37}Cl values (two-point) to the SMOC scale are available in the literature.[28,29,31] Precision (1σ) of the analysis was ≤0.5‰ for δ^{13}C and ≤0.2‰ for δ^{37}Cl.

Evaluation of Isotope Fractionation. Carbon and chlorine isotope ratios of 1,2-DCA were measured at natural abundance and were expressed using the δ-notation (eq 1),

$$\delta^{h}E_{sample} = \frac{R(^{h}E/^{l}E)_{sample}}{R(^{h}E/^{l}E)_{standard}} - 1 \quad (1)$$

where R is the isotope ratio of heavy (^hE) to light (^lE) isotopes of an element E (e.g., $^{13}C/^{12}C$ and $^{37}Cl/^{35}Cl$) and δ values were reported in per mil. The relationship between isotope fractionation and the extent of 1,2-DCA transformation in laboratory experiments was evaluated by a modified form of the Rayleigh distillation eq 2

$$\ln\frac{R_t}{R_0} = \ln\left(\frac{\delta^{h}E_t + 1}{\delta^{h}E_0 + 1}\right) = \varepsilon_{bulk}\cdot\ln f \quad (2)$$

where R_t and R_0 are the current and initial isotope ratios, respectively, and f is the remaining fraction of 1,2-DCA. The compound-average ε_{bulk} values were quantified by least-squares linear regression of eq 2 without forcing the regression through the origin[32] and the uncertainty corresponds to the 95% confidence interval (C.I.) derived from the standard deviation of the regression slope (SI Figure S4). The Rayleigh equation can also be applied to calculate the isotopic fractionation of chlorine despite the higher natural abundance of ^{37}Cl compared to ^{13}C.[33] Calculation of apparent kinetic isotope effects (AKIEs) from estimated ε_{bulk} values is indicated in the SI and their uncertainty was calculated by error propagation. Apparent KIEs that were calculated assuming *stepwise* or *concerted* dihaloelimination are referred hereafter as "$AKIE_{stepwise}$" and "$AKIE_{concerted}$".

RESULTS AND DISCUSSION

Concentration Patterns in Laboratory Experiments. The anaerobic biodegradation batch experiments with *Dehalococcoides* and *Dehalogenimonas*-containing microcosms lasted approximately 9 and 40 days, respectively, at which point most of the initial 1,2-DCA was transformed to ethene (Figure 1). No difference in concentrations and isotope values was observed for the experiments with *Dehalogenimonas*-containing cultures prepared with either acetate or pyruvate as carbon source.

VC was only detected in the *Dehalococcoides*-containing microcosms and the observed product concentration pattern indicates that ethene and VC were formed in parallel reaction pathways. The low level of VC that accumulated (less than 6% of the initial 1,2-DCA added) was subsequently reduced to ethene as indicated by the fact that VC concentration values were lower than in the model prediction (Figure 1c). VC may also be produced abiotically by dehydrohalogenation of 1,2-DCA in alkaline aqueous solution, while hydrolysis of 1,2-DCA to ethylene glycol ($HOCH_2-CH_2OH$) is the predominant pathway under neutral conditions.[34,35] The reported abiotic transformation rate of 1,2-DCA in water, that is, half-life of ∼70 years at 25 °C and pH 7,[34,35] is very low compared to the 1,2-DCA transformation rates observed in this study (Figure 1 and SI), however, indicating that insignificant 1,2-DCA degradation occurred due to abiotic reactions. This is in agreement with the measured 1,2-DCA concentrations in the abiotic controls, which remained within a variation of 5% and 10% of the initial concentration for the experiments with *Dehalococcoides* and *Dehalogenimonas*-containing cultures, respectively. In addition, the average VC concentration in the killed controls of the experiments with *Dehalococcoides*-containing culture represented less than 10% of the maximum amount observed in the live bottles. Small amounts of VC present in killed controls (data not shown) were attributable to remains transferred with the inoculum from previous degradation activity in the enrichment culture (see above).

Cl and C Isotope Fractionation of 1,2-DCA in Laboratory Experiments. Chlorine isotope values of 1,2-DCA showed a trend to more positive values (Figure 2a−b) reflecting an enrichment in ^{37}Cl over ^{35}Cl during dihaloelimination (eq 1). For both cultures, the δ^{37}Cl values were well-described by a Rayleigh isotope fractionation trend (r^2 > 0.98, eq 2, SI Figure S4). However, determined chlorine isotopic fractionation (ε^{Cl}_{bulk}) for the *Dehalococcoides*-containing microcosms (−5.1 ± 0.1‰) was much smaller than for those containing *Dehalogenimonas* (−12.0 ± 0.8‰). The ε^{Cl}_{bulk} values for anaerobic dihaloelimination are higher and much more variable compared to those previously determined in aerobic biodegradation experiments (a) during hydrolytic dehalogenation of 1,2-DCA (C−Cl bond cleavage, Scheme 1b) by *A. aquaticus* and *X. autotrophicus* (−4.4 ± 0.2 and −4.2 ± 0.1‰, respectively) and (b) during oxidation of 1,2-DCA (C−H bond cleavage, Scheme 1a) by *Pseudomonas* sp. (−3.8 ± 0.2‰).[28]

In a similar way as observed for chlorine, the δ^{13}C values during dihaloelimination of 1,2-DCA (Figure 2c, d) also showed an enrichment in ^{13}C over ^{12}C which followed a Rayleigh trend (r^2 > 0.98). However, in contrast to chlorine, the ε^{C}_{bulk} value for the *Dehalococcoides*-containing microcosms

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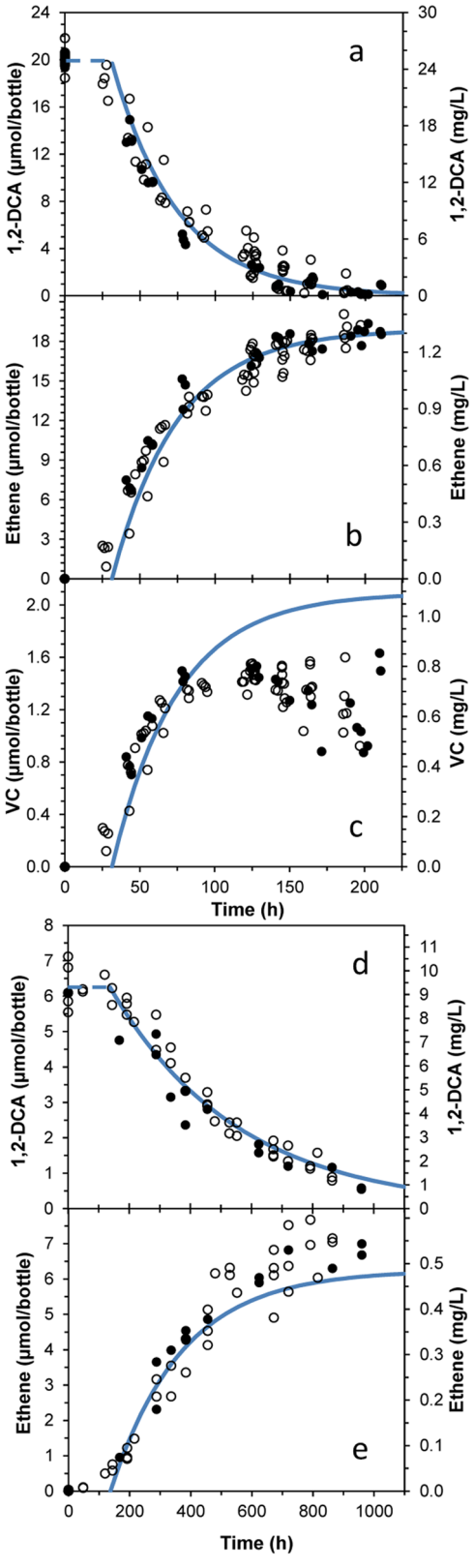

Figure 1. Biodegradation of 1,2-DCA and accumulation of daughter products in batch experiments with *Dehalococcoides*(a−c) and *Dehalogenimonas* (d, e) containing microcosms. For the experiments with *Dehalogenimonas*-containing culture, data from the microcosms with acetate or pyruvate are combined. Open circles represent data points collected by GC headspace measurement on a single bottle that continued to be incubated (headspace monitoring); closed circles indicate those bottles that were sacrificed for isotopic analysis

Figure 1. continued

immediately after the GC measurement. The left ordinate shows the total amounts per bottle and directly reveals the stoichiometry of 1,2-DCA conversion to ethene and VC (not detected in the *Dehalogenimonas*-containing microcosms); aqueous phase concentrations that take into account partitioning to the headspace are shown on the right ordinate. Blue lines represent first-order fits according to SI eqs S3−S5.

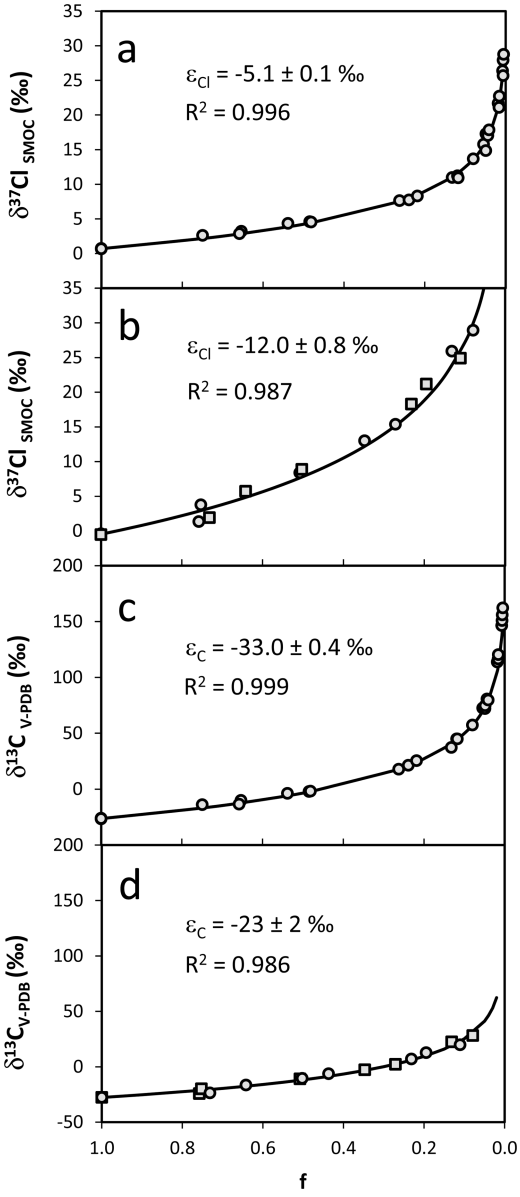

Figure 2. Chlorine and carbon isotopic composition of 1,2-DCA during biodegradation by *Dehalococcoides* (a, c) and *Dehalogenimonas*-containing cultures (b, d); f is the fraction of 1,2-DCA remaining. The error bars for isotope values are smaller than the symbols. For the experiments with *Dehalogenimonas*-containing culture, data from the microcosms with acetate (circles) or pyruvate (squares) are combined. The lines are models fit to isotope data according to eq 2.

(−33.0 ± 0.4‰) was larger than for those containing *Dehalogenimonas* (−23 ± 2‰). For both microbial cultures, carbon isotope fractionation was much higher than for chlorine, in agreement with the large primary carbon isotope effects

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expected for C−Cl bond cleavage.[36] The observed differences of chlorine and carbon isotope fractionation during dihaloelimination of 1,2-DCA by enrichment cultures harboring distinct bacteria are further discussed below. The δ^{37}Cl and δ^{13}C values of 1,2-DCA in the controls did not change significantly during both experiments (i.e., + 0.7 ± 0.1‰ and −0.5 ± 0.1‰, n = 5, and −26.4 ± 0.2‰ and −27.2 ± 0.2‰, n = 5, respectively).

For microbial dihaloelimination of 1,2-DCA, several ε^{C}_{bulk} values from previous laboratory studies are available for comparison. The ε^{C}_{bulk} determined in this study for the *Dehaloccoides*-containing culture (−33.0 ± 0.4‰) agrees well with that determined by Hunkeler et al.[21] in a laboratory microcosm prepared with soil and groundwater from a contaminated site (−32 ± 1‰) and with those obtained by Schmidt et al.[14] using two pure *Dehalococcoides* strains (−31 ± 1‰ and −29 ± 3‰ for *D. mccartyi* strains BTF08 and 195, respectively). In contrast, our first measurement of an ε^{C}_{bulk} for a *Dehalogenimonas*-containing culture resulted in a significantly lower value of −23 ± 2‰. Even lower ε^{C}_{bulk} values (−16.7 ± 0.5‰ and −7.3 ± 0.2‰) were measured by Hirschorn et al.[22] in different anaerobic enrichment cultures originating from contaminated sites. These authors hypothesized that different enzymes or enzymatic reaction pathways (*stepwise* versus *concerted* dihaloelimination) may control isotopic fractionation during 1,2-DCA dihaloelimination. In addition, if rate-limiting steps preceding dehalogenation occur, the observable isotope effect will be smaller (i.e., masked) compared to the intrinsic isotope effect.[23]

With the exception of the two relatively low values determined by Hirschorn et al.,[22] the ε^{C}_{bulk} values for anaerobic dihaloelimination of 1,2-DCA are within a similar range compared to those reported for aerobic hydrolytic dehalogenation in previous studies, from −21.5 to −33.0‰ (average of −29 ± 3‰, ± 1σ, n = 24).[19,28,37,38] Also for anaerobic oxidation of 1,2-DCA under nitrate reducing conditions, Hirschorn et al.[39] measured an ε^{C}_{bulk} of −26 ± 4‰, suggesting again that 1,2-DCA biotransformation occurs via hydrolytic dehalogenation. Therefore, based on carbon isotope fractionation alone it would be difficult to distinguish between aerobic hydrolytic and anaerobic reductive degradation. In addition, in the field it is not possible to determine the extent of mass removal that is uniquely caused by degradation and, consequently, ε^{C}_{bulk} values cannot be evaluated. Hence, a certain extent of observable carbon isotope fractionation in the field (i.e., $\Delta\delta^{13}$C) could have been caused by a strongly isotope fractionating reaction that has proceeded little, or a weakly isotope fractionating reaction that has proceeded further. For example, much lower ε^{C}_{bulk} values were reported for aerobic oxidation (C−H bond cleavage), from −3.0 to −5.3‰ (average of −3.8 ± 0.8‰, ± 1σ, n = 6).[19,28] In order to learn something about the *kind* of process (i.e., aerobic vs anaerobic biodegradation) the magnitude of isotope fractionation of one element alone is, hence, not sufficient, but it must be considered relative to that of another. Analysis of a second element is, therefore, necessary to resolve the issue.

Dual C−Cl Isotope Patterns in the Laboratory. Carbon and chlorine δ isotope values of 1,2-DCA from the experiments with *Dehalococcoides* and *Dehalogenimonas*-containing cultures were combined in a dual element isotope plot resulting in linear trends ($r^2 \geq 0.997$) with strongly distinct slopes ($\Lambda = \Delta\delta^{13}C/\Delta\delta^{37}Cl \approx \varepsilon^{C}_{bulk}/\varepsilon^{Cl}_{bulk}$, stated together with 95% confidence intervals, Figure 3a). For *Dehalococcoides*-containing microcosms, a much larger Λ value (6.8 ± 0.2) than that of those

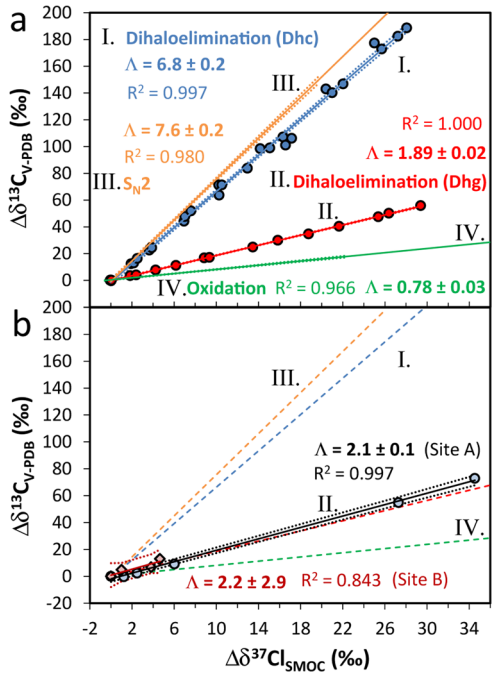

Figure 3. (a) Dual C−Cl isotope patterns during biodegradation of 1,2-DCA in the laboratory. Circles are data points for anaerobic dihaloelimination obtained in this study. For the experiments with *Dehalogenimonas*-containing culture, data from the microcosms with acetate or pyruvate are combined. Trend lines determined for aerobic degradation pathways (oxidation and hydrolytic dehalogenation via S_N2) in a previous study are also indicated;[28] these trend lines were slightly extrapolated for better comparison. (b) Field isotope data and dual C−Cl isotope patterns from site A (circles) and B (rhombus). Dashed lines represent the trend lines determined for aerobic and anaerobic degradation pathways in laboratory experiments. In both panels (a, b), dotted lines indicate the 95% confidence intervals of the linear regression, error bars of $\Delta\delta^{13}$C and $\Delta\delta^{37}$Cl values are smaller than the symbols and Λ values (±95% C.I.) are given by the slope of the linear regressions.

with *Dehalogenimonas* (1.89 ± 0.02) was observed as a result of the much lower ε^{Cl}_{bulk} and higher ε^{C}_{bulk} values (−5.1 ± 0.1‰ and −33.0 ± 0.4‰, respectively, compared to −12.0 ± 0.8‰ and −23 ± 2‰, respectively). This result suggests differences in 1,2-DCA enzymatic dehalogenation by *Dehalococcoides* and *Dehalogenimonas*-containing cultures. The interpretation is reinforced by different daughter compounds during 1,2-DCA transformation by the distinct microbial cultures, that is, formation of ethene and VC in the experiments with *Dehalococcoides*-containing culture, but only ethene with *Dehalogenimonas*-containing culture. Potential reasons for the distinctly different Λ values during anaerobic dihaloelimination of 1,2-DCA are further discussed below.

The isotope patterns observed during anaerobic dihaloelimination of 1,2-DCA were compared with those determined for aerobic biodegradation pathways of 1,2-DCA by Palau et al.[28] (Figure 3a). For the experiments with the *Dehalogenimonas*-containing culture, the Λ value (1.89 ± 0.02) is much lower than the one of hydrolytic dehalogenation (7.6 ± 0.2, via S_N2) but significantly higher than for aerobic oxidation (0.78 ± 0.03). Compared to *Dehalogenimonas*-containing microcosms, the Λ value obtained for those containing *Dehalococcoides* (6.8 ± 0.2) is also higher than for aerobic oxidation and closer to, but significantly different (ANCOVA, p < 0.0001) from hydrolytic dehalogenation (7.6 ± 0.2). These results are further

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discussed below for their ability to differentiate between aerobic and anaerobic biodegradation pathways of 1,2-DCA in the environment using actual field data.

Potential Reaction Mechanisms. The different Λ values observed for dihaloelimination by cultures harboring different bacteria might be interpreted as a result of different reaction pathways (i.e., *stepwise* vs *concerted*). A closer look at the underlying isotope effects, however, allows addressing this question in more detail.

Isotopic fractionation values and derived AKIEs from abiotic reactions are often considered closest to the intrinsic isotope effects.[40] Therefore, AKIEs reported in previous abiotic degradation studies of chlorinated ethanes (see details in SI) were used for comparison. Estimated $AKIE^{C}_{stepwise}$ values for dihaloelimination of 1,2-DCA by *Dehalococcoides* (1.0707 ± 0.0009) and *Dehalogenimonas* (1.048 ± 0.004) containing cultures in this study were clearly above the range reported for abiotic *stepwise* dihaloelimination (from 1.0212 ± 0.0005 to 1.037 ± 0.001, see SI),[20,41] suggesting that a *concerted* mechanism involving both C−Cl bonds in the initial transformation step is more likely ($AKIE^{C}_{concerted}$ of 1.0341 ± 0.0004 and 1.024 ± 0.003 for *Dehalococcoides* and *Dehalogenimonas*-containing cultures, respectively). These values agreed well with reported $AKIEs^{C}_{concerted}$ of 1.03 and 1.023 for abiotic dihaloelimination of 1,2-DCA by Zn(0)[48] and microbial dihaloelimination of 1,2-dichloropropane by *Dehalogenimonas*,[29] respectively, whereas they would be consistent with both, a stepwise ($AKIE^{C}_{stepwise}$ = 1.033) or a concerted ($AKIE^{C}_{concerted}$ = 1.017) mechanism considered to interpret observations by Fletcher et al.[42] during microbial dihaloelimination of 1,2-dichloropropane by *Dehalococcoides* populations.

However, similar Λ values would be expected for the same reaction mechanism and, therefore, the observed difference suggests that despite the evidence of a *concerted* dihaloelimination of 1,2-DCA for both experiments with different microbial cultures, this concerted mechanism must be realized in different ways in both cultures. Recent studies on tetrachloroethylene enzymatic reductive dechlorination[43,44] postulated that similar dehalogenation reactions could result in different Λ values due to a shift of the rate-limiting step within a reaction sequence. This step, prior to the dehalogenation step, would become rate-limiting and mask the intrinsic primary isotope effect during C−Cl bond cleavage. In addition, if this step produce a small but non-negligible isotope fractionation (e.g., during binding of substrate to the enzyme), it could lead to a different Λ value. However, this explanation is not consistent with the results of this study: (i) large C and Cl isotope effects reflecting C−Cl bond transformation in both experiments; (ii) large difference of isotope effects between the experiments with *Dehalococcoides* and *Dehalogenimonas*-containing cultures (i.e., ε_{bulk} values 7 and 10‰ apart for Cl and C, respectively); (iii) countertrends in these isotope effects (i.e., ε^{C}_{bulk} was greater with the *Dehalococcoides*, but ε^{Cl}_{bulk} greater with the *Dehalogenimonas*-containing culture) and (iv) detection of different daughter products in the microcosms with different cultures. These lines of evidence suggest that the observed differences on ε_{bulk} and Λ values between the experiments with cultures harboring different bacteria were associated with a different manner and order of bond breakage (e.g., synchronous/asynchronous or the way how leaving groups were stabilized in different enzyme environments) rather than other reasons invoked previously (i.e., binding, mass transfer). Specifically, Payne et al. recently proposed reduction of the halogenated substrate via halogen-cobalt bond formation in microbial reductive dehalogenases.[45] This new mechanism suggests that a reason for the observed differences in C and Cl isotope effects between both cultures might be a distinct interaction mode between cobalamin dependent enzymes and 1,2-DCA. For reductive dehalogenases catalyzing dihaloelimination, Payne et al. proposed that formation of the Co-halogen bond occurs concomitant with leaving of the vicinal halogen atom.[45] Comparison of the isotope effects observed in this study with those obtained using enzyme extracts, corrinoids and theoretical quantum mechanical/molecular mechanical (QM/MM) modeling in future studies may help to elucidate the reaction mechanisms and the formation of VC in greater detail.

Cl and C Isotope Ratios of 1,2-DCA in Field Samples. The 1,2-DCA concentrations in groundwater samples analyzed for isotope ratios (SI Table S2) range between 2410 and 0.03 mg/L at site A (SI Figure S2) and from 1810 to 0.004 mg/L at site B (SI Figure S3). At site A, samples with relatively low concentrations (<1 mg/L) show isotopic compositions more enriched in heavy isotopes (from +28.4 to +35.6‰ for δ^{37}Cl and from +33.5 to +51.5‰ for δ^{13}C) compared to those with higher concentrations (from +1.1 to +7.1‰ for δ^{37}Cl and from −21.7 to −12.2‰ for δ^{13}C). Similarly, at site B, the highest isotope ratios of 1,2-DCA, up to +28.9‰ for δ^{37}Cl (only chlorine was measured in this sample) and −5.5‰ for δ^{13}C, were also measured in samples with concentrations <1 mg/L (SI Table S2), strongly suggesting the occurrence of 1,2-DCA biodegradation at both sites.

A linear correlation between δ^{37}Cl and δ^{13}C of 1,2-DCA in groundwater samples was obtained for both sites (Figure 3b), confirming that transformation of 1,2-DCA is an important process in the subsurface. The Λ values determined for site A (2.1 ± 0.1, r^2 = 0.997) and B (2.2 ± 2.9, r^2 = 0.84) are the same within the uncertainty. These field Λ values are strongly distinct compared to those measured in the laboratory for aerobic biodegradation pathways of 1,2-DCA (i.e., hydrolytic dehalogenation and C−H bond oxidation, Figure 3b), which agrees with the reducing conditions observed at both sites. The field Λ values are much closer to the one determined for 1,2-DCA anaerobic dihaloelimination by the *Dehalogenimonas*-containing culture (1.89 ± 0.02) compared to that measured for the *Dehalococcoides*-containing microcosms (6.8 ± 0.2). Taking into account the uncertainty of measurements in the field, additional data from contaminated sites under different geochemical conditions will provide the information needed to test and strengthen further the use of the dual isotope approach to evaluate the pathways involved in biodegradation of 1,2-DCA.

In addition, the dual isotope approach may help to select the right ε_{bulk} value for calculating approximately the extent of 1,2-DCA biodegradation in the field, taking into account that the Rayleigh equation applied to field samples tends to underestimate the actual degree of biodegradation.[46] For example, at site A and B maximal shifts in carbon isotope ratios (i.e., $\Delta\delta^{13}C_{max}$) of 73.2 ± 0.7 and 13.0 ± 0.7‰ were observed, respectively (SI Table S2). The ε^{C}_{bulk} value obtained from anaerobic dihaloelimination of 1,2-DCA by *Dehalogenimonas*-containing culture was used due to the similar Λ value (Figure 3b), resulting in 1,2-DCA remaining fractions of 4.3 ± 1.2% (site A) and 56 ± 3% (site B) according to the Rayleigh equation (eq 2, the uncertainty was estimated by error propagation). It is important to note that using the ε^{C}_{bulk} value from the experiments with *Dehalococcoides*-containing

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culture significantly higher values would be obtained: 11.2 ± 0.4% (site A) and 67 ± 2% (site B). Estimated remaining fractions of 1,2-DCA would be even more different (<4% in both sites) if the average ε^{C}_{bulk} value associated with aerobic oxidation of 1,2-DCA was used (−3.8 ± 0.8‰, ± 1σ, n = 6),[19,28] leading to a critically overestimated extent of biodegradation. This example illustrates how uncertainties in the assessment of natural biodegradation at field sites can be significantly reduced if a dual element isotope approach is pursued.

Environmental Significance. This study illustrates the potential of a dual C−Cl isotope approach to investigate biodegradation of 1,2-DCA in the field. Groundwater contaminant plumes are dynamic and highly heterogeneous systems subject to temporal and spatial geochemical variations that control biodegradation processes in the aquifer.[47] Gossett (2010) showed that *aerobic* VC oxidation was sustained at dissolved oxygen (DO) concentrations below 0.02 mg/L.[48] Such low DO concentrations are typically considered to indicate *anoxic* conditions in the field[48] and, therefore, this can lead to a mischaracterization of biodegradation conditions in contaminated sites. Hence, additional tools are necessary for a better characterization of chlorinated contaminants biodegradation in the environment. For 1,2-DCA, dual C−Cl isotope analysis may allow identification of either *aerobic* or *anaerobic* ongoing biodegradation in groundwater. In addition, changes in redox conditions along the plume (e.g., from reducing conditions at the source area to oxic conditions at the fringe zone) could result in two different dual C−Cl isotope correlations in sequence, for example, anaerobic dihaloelimination and aerobic oxidation, respectively. Based on the results of this study, dual element isotope fractionation trends of 1,2-DCA can be expected to detect such a change in transformation pathways.

In addition, the different Λ values determined for *Dehalococcoides* and *Dehalogenimonas*-containing enrichment cultures in the laboratory suggest that a dual isotope approach might even be useful to characterize degradation by different bacteria if the same apparent pathway prevails (i.e., dihaloelimination of 1,2-DCA). On one hand, this could help to identify the microbial community responsible for reductive dechlorination of 1,2-DCA in the field. On the other hand, in addition to *Dehalococcoides* and *Dehalogenimonas*, other anaerobic dehalorespiring bacteria are able to reductively dechlorinate 1,2-DCA, including *Dehalobacter*[7] and *Desulfitobacterium*.[49] The present study is, therefore, a first step and the Λ values determined from 1,2-DCA dihaloelimination by *Dehalococcoides* and *Dehalogenimonas*-containing enrichment cultures should be compared to data obtained with other types of microbes in future biodegradation studies. Nonetheless, the present study can already contribute to the urgent need of reducing uncertainties in the quantification of biodegradation in the field. The estimation of the extent of contaminant transformation using isotope data is one of the main applications of compound-specific isotope analysis to field studies.[17,18,50] Here, we show that identification of dual element isotope patterns under different conditions (i.e., *oxic* vs *anoxic*) can facilitate the elucidation of the active degradation pathway in the field and the corresponding choice of the appropriate ε_{bulk} value for quantification of degradation.

In particular, even though anaerobic dihaloelimination by the *Dehalococcoides*-containing culture and aerobic hydrolytic dehalogenation (via S_N2) have relatively close Λ values (Figure 3a), which may be difficult to distinguish in the environment due to the uncertainty often associated with field data, both reactions have similar ε^{C}_{bulk} values. The present study, therefore, shows that the dual C−Cl isotope approach is able to distinguish between reactions with small (i.e., oxidation) and large (i.e., hydrolytic dehalogenation and dihaloelimination) ε^{C}_{bulk} values. Hence, it illustrates the prospect of identifying the right ε^{C}_{bulk} value, even if the dehalogenation reaction cannot uniquely be pinned down. In summary, dual C−Cl isotope analysis can be a valuable tool for gaining insight into biodegradation of 1,2-DCA under different environmental conditions in field studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b04998.

Further information about field sites, microbial enrichment cultures, experiments set up details, analytical methods, reaction kinetics, Rayleigh isotope plots, and calculation of AKIEs is available (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) ATSDR Toxicological Profile for 1,2-Dichloroethane. http://www.atsdr.cdc.gov/tfacts38.pdf (accessed June 7, 2013).

(2) Falta, R. W.; Bulsara, N.; Henderson, J. K.; Mayer, R. A. Leaded-gasoline additives still contaminate groundwater. *Environ. Sci. Technol.* **2005**, *39* (18), 379A−384A.

DOI: 10.1021/acs.est.6b04998
Environ. Sci. Technol. 2017, 51, 2685−2694

(3) Hage, J. C.; Hartmans, S. Monooxygenase-mediated 1,2-dichloroethane degradation by Pseudomonas sp. strain DCA1. *Appl. Environ. Microbiol.* **1999**, *65* (6), 2466−70.
(4) van den Wijngaard, A. J.; van der Kamp, K. W.; van der Ploeg, J.; Pries, F.; Kazemier, B.; Janssen, D. B. Degradation of 1,2-dichloroethane by Ancylobacter aquaticus and other facultative methylotrophs. *Appl. Environ. Microbiol.* **1992**, *58* (3), 976−83.
(5) Janssen, D. B.; Scheper, A.; Dijkhuizen, L.; Witholt, B. Degradation of halogenated aliphatic compounds by Xanthobacter autotrophicus GJ10. *Appl. Environ. Microbiol.* **1985**, *49* (3), 673−7.
(6) Klecka, G. M.; Carpenter, C. L.; Gonsior, S. J. Biological transformations of 1,2-dichloroethane in subsurface soils and groundwater. *J. Contam. Hydrol.* **1998**, *34* (1−2), 139−154.
(7) Grostern, A.; Edwards, E. A. Characterization of a Dehalobacter Coculture That Dechlorinates 1,2-Dichloroethane to Ethene and Identification of the Putative Reductive Dehalogenase Gene. *Appl. Environ. Microbiol.* **2009**, *75* (9), 2684−2693.
(8) Egli, C.; Scholtz, R.; Cook, A. M.; Leisinger, T. Anaerobic dechlorination of tetrachloromethane and 1,2-dichloroethane to degradable products by pure cultures of Desulfobacterium sp. and Methanobacterium sp. *FEMS Microbiol. Lett.* **1987**, *43* (3), 257−261.
(9) Yu, R.; Peethambaram, H. S.; Falta, R. W.; Verce, M. F.; Henderson, J. K.; Bagwell, C. E.; Brigmon, R. L.; Freedman, D. L. Kinetics of 1,2-Dichloroethane and 1,2-Dibromoethane Biodegradation in Anaerobic Enrichment Cultures. *Appl. Environ. Microbiol.* **2013**, *79* (4), 1359−1367.
(10) Holliger, C.; Schraa, G.; Stams, A. J. M.; Zehnder, A. J. B. Reductive dechlorination of 1,2-dichloroethane and chloroethane by cell suspensions of methanogenic bacteria. *Biodegradation* **1990**, *1* (4), 253−261.
(11) van der Zaan, B.; de Weert, J.; Rijnaarts, H.; de Vos, W. M.; Smidt, H.; Gerritse, J. Degradation of 1,2-dichloroethane by microbial communities from river sediment at various redox conditions. *Water Res.* **2009**, *43* (13), 3207−16.
(12) Chen, C.; Puhakka, J. A.; Ferguson, J. F. Transformations of 1,1,2,2-Tetrachloroethane under Methanogenic Conditions. *Environ. Sci. Technol.* **1996**, *30* (2), 542−547.
(13) Maymo-Gatell, X.; Anguish, T.; Zinder, S. H. Reductive dechlorination of chlorinated ethenes and 1,2-dichloroethane by "Dehalococcoides ethenogenes" 195. *Appl. Environ. Microbiol.* **1999**, *65* (7), 3108−3113.
(14) Schmidt, M.; Lege, S.; Nijenhuis, I. Comparison of 1,2-dichloroethane, dichloroethene and vinyl chloride carbon stable isotope fractionation during dechlorination by two Dehalococcoides strains. *Water Res.* **2014**, *52*, 146−54.
(15) USEPA. Priority pollutants. http://water.epa.gov/scitech/methods/cwa/pollutants.cfm (accessed January 7, 2015),.
(16) Sun, B. L.; Griffin, B. M.; Ayala-del-Rio, H. L.; Hashsham, S. A.; Tiedje, J. M. Microbial dehalorespiration with 1,1,1-trichloroethane. *Science* **2002**, *298* (5595), 1023−1025.
(17) Thullner, M.; Centler, F.; Richnow, H. H.; Fischer, A. Quantification of organic pollutant degradation in contaminated aquifers using compound specific stable isotope analysis - Review of recent developments. *Org. Geochem.* **2012**, *42* (12), 1440−1460.
(18) Aelion, C. M.; Hohëner, P.; Hunkeler, D.; Aravena, R. *Environmental Isotopes in Biodegradation and Bioremediation*; CRC Press: Boca Raton, 2010; p xiv.
(19) Hirschorn, S. K.; Dinglasan, M. J.; Elsner, M.; Mancini, S. A.; Lacrampe-Couloume, G.; Edwards, E. A.; Lollar, B. S. Pathway dependent isotopic fractionation during aerobic biodegradation of 1,2-dichloroethane. *Environ. Sci. Technol.* **2004**, *38* (18), 4775−4781.
(20) Elsner, M.; Cwiertny, D. M.; Roberts, A. L.; Lollar, B. S. 1,1,2,2-tetrachloroethane reactions with OH-, Cr(II), granular iron, and a copper-iron bimetal: Insights from product formation and associated carbon isotope fractionation. *Environ. Sci. Technol.* **2007**, *41* (11), 4111−4117.
(21) Hunkeler, D.; Aravena, R.; Cox, E. Carbon isotopes as a tool to evaluate the origin and fate of vinyl chloride: laboratory experiments and modeling of isotope evolution. *Environ. Sci. Technol.* **2002**, *36* (15), 3378−84.
(22) Hirschorn, S. K.; Grostern, A.; Lacrampe-Couloume, G.; Edwards, E. A.; Mackinnon, L.; Repta, C.; Major, D. W.; Sherwood Lollar, B. Quantification of biotransformation of chlorinated hydrocarbons in a biostimulation study: added value via stable carbon isotope analysis. *J. Contam. Hydrol.* **2007**, *94* (3−4), 249−60.
(23) Renpenning, J.; Rapp, I.; Nijenhuis, I. Substrate Hydrophobicity and Cell Composition Influence the Extent of Rate Limitation and Masking of Isotope Fractionation during Microbial Reductive Dehalogenation of Chlorinated Ethenes. *Environ. Sci. Technol.* **2015**, *49* (7), 4293−301.
(24) Elsner, M. Stable isotope fractionation to investigate natural transformation mechanisms of organic contaminants: principles, prospects and limitations. *J. Environ. Monit.* **2010**, *12* (11), 2005−2031.
(25) Tobler, N. B.; Hofstetter, T. B.; Schwarzenbach, R. P. Carbon and Hydrogen Isotope Fractionation during Anaerobic Toluene Oxidation by Geobacter metallireducens with Different Fe(III) Phases as Terminal Electron Acceptors. *Environ. Sci. Technol.* **2008**, *42* (21), 7786−7792.
(26) Thullner, M.; Fischer, A.; Richnow, H. H.; Wick, L. Y. Influence of mass transfer on stable isotope fractionation. *Appl. Microbiol. Biotechnol.* **2013**, *97* (2), 441−52.
(27) Fischer, A.; Theuerkorn, K.; Stelzer, N.; Gehre, M.; Thullner, M.; Richnow, H. H. Applicability of stable isotope fractionation analysis for the characterization of benzene biodegradation in a BTEX-contaminated aquifer. *Environ. Sci. Technol.* **2007**, *41* (10), 3689−96.
(28) Palau, J.; Cretnik, S.; Shouakar-Stash, O.; Hoche, M.; Elsner, M.; Hunkeler, D. C and Cl Isotope Fractionation of 1,2-Dichloroethane Displays Unique delta(13)C/delta(37)Cl Patterns for Pathway Identification and Reveals Surprising C-Cl Bond Involvement in Microbial Oxidation. *Environ. Sci. Technol.* **2014**, *48* (16), 9430−7.
(29) Martin-Gonzalez, L.; Hatijah Mortan, S.; Rosell, M.; Parlade, E.; Martinez-Alonso, M.; Gaju, N.; Caminal, G.; Adrian, L.; Marco-Urrea, E. Stable Carbon Isotope Fractionation During 1,2-Dichloropropane-to-Propene Transformation by an Enrichment Culture Containing Dehalogenimonas Strains and a dcpA Gene. *Environ. Sci. Technol.* **2015**, *49* (14), 8666−74.
(30) Aeppli, C.; Holmstrand, H.; Andersson, P.; Gustafsson, O. Direct Compound-Specific Stable Chlorine Isotope Analysis of Organic Compounds with Quadrupole GC/MS Using Standard Isotope Bracketing. *Anal. Chem.* **2010**, *82* (1), 420−426.
(31) Shouakar-Stash, O.; Drimmie, R. J.; Zhang, M.; Frape, S. K. Compound-specific chlorine isotope ratios of TCE, PCE and DCE isomers by direct injection using CF-IRMS. *Appl. Geochem.* **2006**, *21* (5), 766−781.
(32) Scott, K. M.; Lu, X.; Cavanaugh, C. M.; Liu, J. S. Optimal methods for estimating kinetic isotope effects from different forms of the Rayleigh distillation equation. *Geochim. Cosmochim. Acta* **2004**, *68* (3), 433−442.
(33) Elsner, M.; Hunkeler, D. Evaluating chlorine isotope effects from isotope ratios and mass spectra of polychlorinated molecules. *Anal. Chem.* **2008**, *80* (12), 4731−4740.
(34) Jeffers, P. M.; Ward, L. M.; Woytowitch, L. M.; Wolfe, N. L. Homogeneous Hydrolysis Rate Constants for Selected Chlorinated Methanes, Ethanes, Ethenes, and Propanes. *Environ. Sci. Technol.* **1989**, *23* (8), 965−969.
(35) Schwarzenbach, R. P. G. P. M.; Imboden, D. M. *Environmental Organic Chemistry*, 2nd ed. ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 2003; p 1313.
(36) Elsner, M.; Zwank, L.; Hunkeler, D.; Schwarzenbach, R. P. A new concept linking observable stable isotope fractionation to transformation pathways of organic pollutants. *Environ. Sci. Technol.* **2005**, *39* (18), 6896−6916.
(37) Hunkeler, D.; Aravena, R. Evidence of substantial carbon isotope fractionation among substrate, inorganic carbon, and biomass during aerobic mineralization of 1, 2-dichloroethane by Xanthobacter autotrophicus. *Appl. Environ. Microbiol.* **2000**, *66* (11), 4870−6.

DOI: 10.1021/acs.est.6b04998
Environ. Sci. Technol. 2017, 51, 2685−2694

(38) Abe, Y.; Zopfi, J.; Hunkeler, D. Effect of molecule size on carbon isotope fractionation during biodegradation of chlorinated alkanes by Xanthobacter autotrophicus GJ10. *Isot. Environ. Health Stud.* **2009**, *45* (1), 18−26.
(39) Hirschorn, S. K.; Dinglasan-Panlilio, M. J.; Edwards, E. A.; Lacrampe-Couloume, G.; Sherwood Lollar, B. Isotope analysis as a natural reaction probe to determine mechanisms of biodegradation of 1,2-dichloroethane. *Environ. Microbiol.* **2007**, *9* (7), 1651−7.
(40) Lollar, B. S.; Hirschorn, S.; Mundle, S. O.; Grostern, A.; Edwards, E. A.; Lacrampe-Couloume, G. Insights into enzyme kinetics of chloroethane biodegradation using compound specific stable isotopes. *Environ. Sci. Technol.* **2010**, *44* (19), 7498−503.
(41) Hofstetter, T. B.; Reddy, C. M.; Heraty, L. J.; Berg, M.; Sturchio, N. C. Carbon and chlorine isotope effects during abiotic reductive dechlorination of polychlorinated ethanes. *Environ. Sci. Technol.* **2007**, *41* (13), 4662−4668.
(42) Fletcher, K. E.; Loffler, F. E.; Richnow, H. H.; Nijenhuis, I. Stable Carbon Isotope Fractionation of 1,2-Dichloropropane during Dichloroelimination by Dehalococcoides Populations. *Environ. Sci. Technol.* **2009**, *43* (18), 6915−6919.
(43) Badin, A.; Buttet, G.; Maillard, J.; Holliger, C.; Hunkeler, D. Multiple Dual C-Cl Isotope Patterns Associated with Reductive Dechlorination of Tetrachloroethene. *Environ. Sci. Technol.* **2014**, *48* (16), 9179−9186.
(44) Renpenning, J.; Keller, S.; Cretnik, S.; Shouakar-Stash, O.; Elsner, M.; Schubert, T.; Nijenhuis, I. Combined C and Cl isotope effects indicate differences between corrinoids and enzyme (Sulfurospirillum multivorans PceA) in reductive dehalogenation of tetrachloroethene, but not trichloroethene. *Environ. Sci. Technol.* **2014**, *48* (20), 11837−45.
(45) Payne, K. A.; Quezada, C. P.; Fisher, K.; Dunstan, M. S.; Collins, F. A.; Sjuts, H.; Levy, C.; Hay, S.; Rigby, S. E.; Leys, D. Reductive dehalogenase structure suggests a mechanism for B12-dependent dehalogenation. *Nature* **2015**, *517* (7535), 513−6.
(46) Abe, Y.; Hunkeler, D. Does the Rayleigh equation apply to evaluate field isotope data in contaminant hydrogeology? *Environ. Sci. Technol.* **2006**, *40* (5), 1588−1596.
(47) Meckenstock, R. U.; Elsner, M.; Griebler, C.; Lueders, T.; Stumpp, C.; Aamand, J.; Agathos, S. N.; Albrechtsen, H. J.; Bastiaens, L.; Bjerg, P. L.; Boon, N.; Dejonghe, W.; Huang, W. E.; Schmidt, S. I.; Smolders, E.; Sorensen, S. R.; Springael, D.; van Breukelen, B. M. Biodegradation: Updating the concepts of control for microbial cleanup in contaminated aquifers. *Environ. Sci. Technol.* **2015**, *49* (12), 7073−81.
(48) Gossett, J. M. Sustained aerobic oxidation of vinyl chloride at low oxygen concentrations. *Environ. Sci. Technol.* **2010**, *44* (4), 1405−11.
(49) De Wildeman, S.; Diekert, G.; Van Langenhove, H.; Verstraete, W. Stereoselective microbial dehalorespiration with vicinal dichlorinated alkanes. *Appl. Environ. Microbiol.* **2003**, *69* (9), 5643−7.
(50) Braeckevelt, M.; Fischer, A.; Kastner, M. Field applicability of Compound-Specific Isotope Analysis (CSIA) for characterization and quantification of in situ contaminant degradation in aquifers. *Appl. Microbiol. Biotechnol.* **2012**, *94* (6), 1401−1421.
(51) Elsner, M.; Hofstetter, T. B., Current Perspectives on the Mechanisms of Chlorohydrocarbon Degradation in Subsurface Environments: Insight from Kinetics, Product Formation, Probe Molecules, and Isotope Fractionation. In *Aquatic Redox Chemistry*; American Chemical Society, 2011; Vol. *1071*, pp 407−439.

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Environ. Sci. Technol. 2017, 51, 2685−2694