Environmental Science & Technology

Article

pubs.acs.org/est

Hydrogen Isotope Fractionation during the Biodegradation of 1,2-Dichloroethane: Potential for Pathway Identification Using a Multi-element (C, Cl, and H) Isotope Approach

Jordi Palau,[*,†,▽,#] Orfan Shouakar-Stash,[‡,§] Siti Hatijah Mortan,[∥] Rong Yu,[⊥] Monica Rosell,[#] Ernest Marco-Urrea,[∥] David L. Freedman,[⊥] Ramon Aravena,[‡] Albert Soler,[#] and Daniel Hunkeler[†]

[†]Centre for Hydrogeology and Geothermics, University of Neuchâtel, 2000 Neuchâtel, Switzerland

[‡]Department of Earth and Environmental Sciences, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

[§]Isotope Tracer Technologies Inc., Waterloo, Ontario N2 V 1Z5, Canada

[∥]Departament d'Enginyeria Química, Biològica i Ambiental, Universitat Autònoma de Barcelona, Carrer de les Sitges s/n, 08193 Bellaterra, Spain

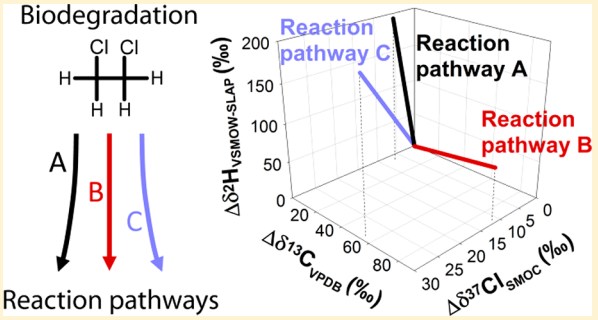
[⊥]Department of Environmental Engineering and Earth Sciences, Clemson University, Clemson, South Carolina 29634, United States

[#]Grup de Mineralogia Aplicada i Geoquímica de Fluids, Departament de Mineralogia, Petrologia i Geologia Aplicada, Facultat de Ciències de la Terra, Universitat de Barcelona, Martí i Franquès s/n, 08028 Barcelona, Spain

[▽]Institute of Environmental Assessment and Water Research (IDAEA), CSIC, and Hydrogeology Group (UPC−CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain

Supporting Information

ABSTRACT: Even though multi-element isotope fractionation patterns provide crucial information with which to identify contaminant degradation pathways in the field, those involving hydrogen are still lacking for many halogenated groundwater contaminants and degradation pathways. This study investigates for the first time hydrogen isotope fractionation during both aerobic and anaerobic biodegradation of 1,2-dichloroethane (1,2-DCA) using five microbial cultures. Transformation-associated isotope fractionation values (ε^{H}_{bulk}) were −115 ± 18‰ (aerobic C−H bond oxidation), −34 ± 4‰ and −38 ± 4‰ (aerobic C−Cl bond cleavage via hydrolytic dehalogenation), and −57 ± 3‰ and −77 ± 9‰ (anaerobic C−Cl bond cleavage via reductive dihaloelimination). The dual-element C−H isotope approach ($\Lambda_{C-H} = \Delta\delta^2H/\Delta\delta^{13}C \approx \varepsilon^{H}_{bulk}/\varepsilon^{C}_{bulk}$, where $\Delta\delta^2H$ and $\Delta\delta^{13}C$ are changes in isotope ratios during degradation) resulted in clearly different Λ_{C-H} values: 28 ± 4 (oxidation), 0.7 ± 0.1 and 0.9 ± 0.1 (hydrolytic dehalogenation), and 1.76 ± 0.05 and 3.5 ± 0.1 (dihaloelimination). This result highlights the potential of this approach to identify 1,2-DCA degradation pathways in the field. In addition, distinct trends were also observed in a multi- (i.e., $\Delta\delta^2H$ versus $\Delta\delta^{37}Cl$ versus $\Delta\delta^{13}C$) isotope plot, which opens further possibilities for pathway identification in future field studies. This is crucial information to understand the mechanisms controlling natural attenuation of 1,2-DCA and to design appropriate strategies to enhance biodegradation.



INTRODUCTION

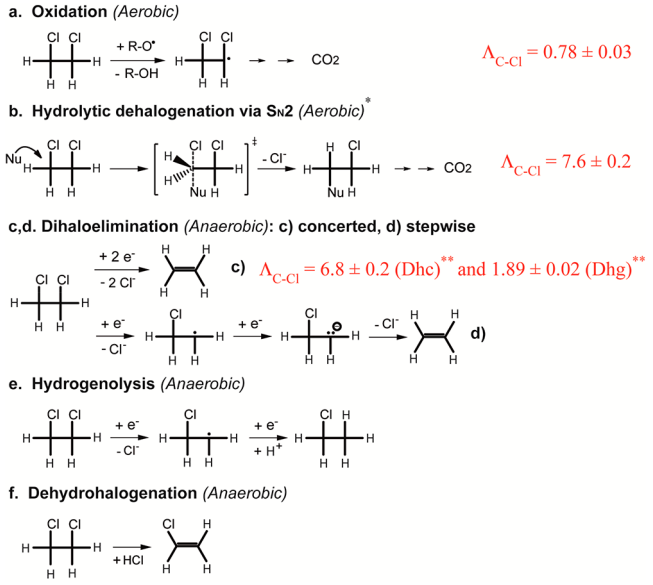
1,2-Dichloroethane (1,2-DCA) is widely used as a chemical intermediate in the industrial production of polyvinyl chloride, as a solvent, and also as a lead scavenger in leaded gasoline.[1] Due to its high production, accidental leakage, and improper disposal, 1,2-DCA has become a prevalent groundwater contaminant. For instance, in 2015, a total of 186 tons of 1,2-DCA (not including on-site land disposal) were released to the environment in the United States,[2] which posed a threat to human and wildlife health due to its high toxicity.[3]

1,2-DCA can undergo biodegradation via distinct degradation pathways under oxic[4−6] and anoxic conditions[7−11] (Scheme 1). Under oxic conditions, 1,2-DCA can be biodegraded by oxidation via a monooxygenase[4] (Scheme 1a) and hydrolytic dehalogenation[5,6] (Scheme 1b). Initial products of both reactions are further degraded to innocuous end products. Under reducing conditions, 1,2-DCA is usually transformed by dihaloelimination to ethene[7,8] (either concerted or stepwise β-elimination; Scheme 1c,d) or hydrogenolysis to chloroethane (CA)[11] (Scheme 1e). In addition, 1,2-DCA can be transformed to vinyl chloride (VC) via dehydrohalogenation

Received: June 6, 2017
Revised: August 14, 2017
Accepted: August 16, 2017
Published: August 16, 2017

DOI: 10.1021/acs.est.7b02906
Environ. Sci. Technol. 2017, 51, 10526−10535

Scheme 1. Biodegradation Pathways of 1,2-DCA in Aqueous Systems and Reported Λ_{C-Cl} Values[14a]



[a]Asterisks indicate that transformation via hydrolytic dehalogenation was also proposed under nitrate-reducing conditions.[18] Double asterisks indicate values obtained from experiments with *Dehalococcoides*- (Dhc) and *Dehalogenimonas*- (Dhg) containing enrichment cultures.[14]

(Scheme 1f) by pure *Dehalococcoides* strains[12,13] and *Dehalococcoides*-containing cultures;[10,14] however, VC is typically detected at much lower concentrations compared to ethene. Therefore, chlorinated products such as CA and VC can accumulate under anoxic conditions. Like 1,2-DCA, both CA and VC are groundwater contaminants and are considered as priority pollutants by the U.S. Environmental Protection Agency (USEPA),[3] emphasizing the need for elucidation of active biodegradation pathways in the field.

Owing to the high susceptibility for 1,2-DCA to be transformed under different redox conditions, the assessment of its fate in the subsurface is not an easy task. On the one hand, aerobic biodegradation of 1,2-DCA might be sustained at very low dissolved oxygen concentrations as shown for VC oxidation.[15] Furthermore, anaerobic oxidation of 1,2-DCA was demonstrated under nitrate-reducing conditions,[16,17] likely via hydrolytic dehalogenation (Scheme 1b).[18] On the other hand, contaminated sites are usually impacted by mixtures of compounds, which complicates pathway identification from analysis of degradation products because the same products can be formed from different precursors. For instance, CA can also be formed from 1,1,1-trichloroethane (1,1,1-TCA)[19] and VC and ethene from trichloroethene (TCE)[12] by reductive dechlorination. In addition, end products of the aerobic degradation pathways of 1,2-DCA, i.e., inorganic carbon and Cl^-, are ubiquitous and often occur at high background concentrations in groundwater. Hence, additional tools are necessary for better characterization of 1,2-DCA biodegradation in the field. This information is essential for (i) evaluating the natural attenuation of 1,2-DCA at contaminated sites and (ii) predicting potential accumulation of toxic products.

Dual carbon and chlorine isotope analysis during substrate transformation is increasingly used to elucidate biodegradation pathways and obtain insight into enzymatic reaction mechanisms for chlorinated ethenes[20−23] and ethanes.[14,24] However, until recently, a multi-element isotope approach including hydrogen isotope data was not feasible because online hydrogen isotope analysis of chlorinated compounds was hampered by the formation of HCl during the high-temperature conversion of chlorinated analytes to H_2.[25] New analytical methods for online compound-specific hydrogen isotope analysis (H−CSIA) by gas chromatography−isotope ratio mass spectrometry (GC−IRMS) were developed that largely circumvent the formation of HCl by the use of a chromium metal reactor interface to form H_2. These new methods were validated using different compounds such as chlorinated ethenes and hexachlorocyclohexane.[26−29] For instance, δ^2H values with a precision better than ±7‰ were obtained for both trichloroethene and *cis*-1,2-dichloroethene (*cis*-DCE) by Shouakar-Stash and Drimmie.[26] H−CSIA studies have so far been applied to only a few chlorinated compounds[30−32] and, to the best of our knowledge, multi-element isotope studies including hydrogen isotope data are currently nonexistent for chlorinated ethanes.

Combined shifts in isotope ratios of two elements (e.g., $\Delta\delta^2$H versus $\Delta\delta^{13}$C) generally exhibit a linear relationship with a slope ($\Lambda_{C-H} = \Delta\delta^2H/\Delta\delta^{13}C$) reflecting the extent of H and C isotope effects, which are controlled by chemical bond breakage or formation. Therefore, different Λ_{C-H} values may be expected for distinct transformation mechanisms involving different elements.[33] For 1,2-DCA, dual C−Cl isotope data were recently reported and different Λ_{C-Cl} values (Scheme 1) were observed during both aerobic[24] and anaerobic[14] biodegradation of 1,2-DCA. However, relatively similar Λ_{C-Cl} values were observed for aerobic hydrolytic dehalogenation by *Xanthobacter autotrophicus* GJ10 and *Ancylobacter aquaticus* AD20 (7.6 ± 0.2) and anaerobic dihaloelimination by a *Dehalococcoides*-containing enrichment culture (6.8 ± 0.2). Taking into account the uncertainty of measurements at contaminated sites, it may be difficult to distinguish the two pathways (i.e., hydrolytic dehalogenation versus dihaloelimination) in field studies solely based on dual C−Cl isotope data. In addition to C and Cl, analysis of H isotope ratios for 1,2-DCA may increase the possibilities for a dual- (C versus H or Cl versus H) or multi-element (C versus Cl versus H) isotope approach for differentiating between hydrolytic dehalogenation and dihaloelimination pathways.

For the dihaloelimination of 1,2-DCA, distinct Λ_{C-Cl} values were reported from microcosm experiments with *Dehalococcoides* (6.8 ± 0.2) and *Dehalogenimonas* (1.89 ± 0.02) containing enrichment cultures and a different mode of concerted bond cleavage rather than two different reaction mechanisms (i.e., stepwise versus concerted) was proposed to explain this difference.[14] However, further insight into the reductive dehalogenation mechanisms of 1,2-DCA may be obtained from hydrogen isotope data. Owing to the usually large hydrogen isotope fractionation (ε^H_{bulk}), secondary hydrogen isotope effects during C−Cl bond breakage might be detected and measured.[34] In this case, the magnitude of secondary ε^H_{bulk} values may vary depending on the reaction mechanism (i.e., stepwise versus concerted dehalogenation).

In this study, hydrogen isotope fractionation during biodegradation of 1,2-DCA via aerobic (oxidation and hydrolytic dehalogenation) and anaerobic (dihaloelimination) degradation pathways was determined for the first time using different pure microbial strains and enrichment cultures in laboratory experiments. 2D (H versus C and H versus Cl) and

DOI: 10.1021/acs.est.7b02906
Environ. Sci. Technol. 2017, 51, 10526−10535

3D (H versus C versus Cl) multi-element isotope approaches were used (i) to characterize the Λ_{C-H} and Λ_{Cl-H} values during biodegradation of 1,2-DCA under different redox conditions; (ii) to determine whether the resultant multi-element isotope patterns are sufficiently different to distinguish between different pathways, particularly between hydrolytic dehalogenation and dihaloelimination pathways; and (iii) to obtain further insight into underlying reaction mechanisms. In addition, for the 3D isotope approach, new procedures were proposed to characterize pathway-specific multi-element (Cl, C, and H) isotope trends, which can be applicable to other multi-element isotope studies with three elements.

MATERIALS AND METHODS

Pure and Enrichment Cultures. A total of three pure strains with known initial biotransformation mechanisms were used for the aerobic experiments: *Pseudomonas* sp. strain DCA1 (oxidation)[4] and *Xanthobacter autotrophicus* GJ10 and *Ancylobacter aquaticus* AD20 (hydrolytic dehalogenation).[5,6] *Pseudomonas* sp. strain DCA1 was kindly provided by E. Edwards (Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON), and *X. autotrophicus* GJ10 (DSMZ 3874) and *A. aquaticus* AD20 (DSMZ 9000) were purchased (DSMZ, Braunschweig, Germany). The growth medium was prepared as described by Hunkeler and Aravena,[35] and further cultivation details are available in Palau et al.[24]

Anaerobic cultures for reductive dihaloelimination experiments were prepared using two enrichment cultures with different bacterial populations, which were characterized in previous studies to determine organohalide-respiring bacteria (ORB) capable of 1,2-DCA degradation.[10,14,36] The growth media used and cultivation details for *Dehalococcoides*- and *Dehalogenimonas*-containing cultures are available in Palau et al.[14]

Batch Experiments Preparation and Sampling. Aerobic biodegradation experiments were performed at the University of Neuchâtel (UN), Switzerland. Microcosm batch tests were prepared in 250 mL glass bottles, which contained 185 mL of medium and were capped with Mininert valves (VICI Precision Sampling, Baton Rouge, LA). Experiments and controls were amended with 22.5 μL of pure 1,2-DCA to produce an initial aqueous concentration of 1.5 mM (when taking into account partitioning between the headspace and liquid using Henry's Law). All experiments were conducted in triplicate. Bottles were shaken upside down to prevent leakage of the gas phase through the valve. For concentration and isotopic analysis, aqueous samples (1.5 mL) were taken from the 250 mL bottles at selected time points and preserved frozen[37] in 2 mL vials with NaN_3 (1 g/L). Abiotic control bottles were prepared with 185 mL of autoclaved mineral medium, and samples were collected and preserved as described for the experimental bottles.

Anaerobic biodegradation experiments with *Dehalococcoides*- and *Dehalogenimonas*-containing cultures were performed at Clemson University (CU) and at the Universitat Autònoma de Barcelona (UAB), respectively. Microcosms were prepared in anoxic chambers and the bottles (120 mL total volume) were sealed with Teflon-faced rubber septa and aluminum crimp caps to maintain anoxic conditions.

For the batch tests with *Dehalococcoides*-containing culture, 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) to produce an initial aqueous-phase concentration of ~0.25 mM. 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).[10] Killed controls were prepared by adding phosphoric acid to the bottles, followed by the 1,2-DCA.

For the experiments with *Dehalogenimonas*-containing culture, a total of 16 serum bottles were prepared by dispensing 65 mL of a sterilized anoxic medium described elsewhere;[36] however, in half of the bottles, pyruvate (5 mM) was replaced by acetate (5 mM) as carbon source. The microcosms were inoculated with 3 mL of the *Dehalogenimonas*-containing culture and 1,2-DCA was added from a stock solution in acetone to give an initial aqueous-phase concentration of ~0.1 mM. Abiotic control bottles containing the growth medium with 1,2-DCA but without inoculum were prepared as described for the experimental bottles. In addition, live controls without 1,2-DCA were prepared to account for the transfer of compounds from previous degradation experiments with the inoculum.

Isotopic and Concentration Analysis. A detailed description of analytical methods and equipment used for the isotopic and concentration analysis is available in the Supporting Information. Online H−CSIA of 1,2-DCA was performed at Isotope Tracer Technologies Inc., Canada, according to Shouakar-Stash and Drimmie.[26] Briefly, measurements of hydrogen isotope ratios for 1,2-DCA at natural abundance were determined by GC−IRMS equipped with a chromium reduction system. The instrument was tuned and the H_3^+ factor was determined every day before analysis of standards and samples. The H_3^+ factor usually ranged between 4.5 and 5.5. Isotope ratios were reported using the δ-notation (eq 1):

$$\delta^2H_{sample} = \frac{R_{sample}}{R_{standard}} - 1 \quad (1)$$

where $R = {}^2H/{}^1H$, corresponding to the ratio of m/z 3 ($^2H^1H$) to m/z 2 ($^1H^1H$) measured in separate Faraday caps. The δ-values were expressed in per mil (1 ‰ = 1 mUr), and the notations $\delta^2H_{VSMOW-SLAP}$ and $\Delta\delta^2H_{VSMOW-SLAP}$ were used to indicate δ-values calibrated to the VSMOW-SLAP international scale and changes in calibrated δ-values during degradation (i.e., $\Delta\delta^2H = \delta^2H_t - \delta^2H_0$), respectively. Hydrogen isotope ratios were calibrated using 1,2-DCA and TCE as reference compounds in the $\delta^2H_{VSMOW-SLAP}$ range between −50 ± 1‰ and +565 ± 4‰.[27,38] External laboratory standards of 1,2-DCA and TCE were dissolved in water and measured similarly to the samples. Further details about δ^2H values (two-point) calibration to the VSMOW−SLAP scale are available in the Supporting Information. Additional aqueous standards of 1,2-DCA were interspersed in each sample sequence to ensure the stability of the measurements during the course of sample analyses. Samples and standards were diluted to a similar concentration and measured in duplicate. Precision (1σ) of the δ^2H values for 1,2-DCA on the analysis of the standards was <5‰ (n = 54) (see further details in the Supporting Information).

The concentrations of 1,2-DCA were measured by headspace analysis using a GC−mass spectrometer (GC−MS) at UN[24] laboratory (aerobic experiments) and a GC−flame ionization detector (GC−FID) at the CU[10] and UAB[36] laboratories

DOI: 10.1021/acs.est.7b02906
Environ. Sci. Technol. 2017, 51, 10526−10535

(anaerobic experiments). The concentration of 1,2-DCA in the abiotic controls for the aerobic (1.55 ± 0.03 mM, n = 12) and anaerobic experiments (0.257 ± 0.004 mM, n = 5 and 0.094 ± 0.009 mM, n = 6) remained at the initial concentration during the experiments, indicating that compound losses through the caps and abiotic degradation during incubation were insignificant.

Evaluation of Isotope Fractionation. For a given substrate, the relationship between observable compound-average isotope fractionation (ε_{bulk}), and the extent of biotransformation can be described by a modified form of the Rayleigh distillation eq 2 in laboratory experiments:

$$\ln \frac{R_t}{R_0} = \ln\left(\frac{1 + \delta^2H_t}{1 + \delta^2H_0}\right) = \varepsilon^H_{bulk} \times \ln f \quad (2)$$

where the subscripts "t" and "0" refer to the current and initial bulk isotope ratios, respectively, and f is the remaining fraction of the substrate.

For the aerobic experiments, f was corrected for substrate removal by repetitive liquid samples withdrawn from the same batch reactor according to Buchner et al. (see eqs S1 and S2),[39] which also takes into account volatilization of the substrate to the bottle headspace. An aqueous-phase 1,2-DCA concentration decrease of <5% was estimated as a result of the change in the headspace to solution ratio during the aerobic experiments. The correction of f due to mass removal during sampling was not necessary for the anaerobic experiments because these were prepared with numerous parallel replicates, which were sequentially sacrificed for sampling.

ε_{bulk} values were quantified by least-squares linear regression of eq 2 without forcing the regression through the origin (see Figure S1).[40] Uncertainties are represented by 95% confidence intervals (CI). Calculation of position-specific apparent kinetic isotope effects (AKIEs) is indicated in the Supporting Information.

Dual-element isotope fractionation patterns for different degradation pathways were characterized by the slope of the linear regressions in a 2D isotope plot, i.e., $\Lambda_{C-H} = \Delta\delta^2H/\Delta\delta^{13}C$ and $\Lambda_{Cl-H} = \Delta\delta^2H/\Delta\delta^{37}Cl$, ± 95% CI. For each degradation experiment, the observed multi-element (Cl, C, and H) isotope fractionation trend in a 3D isotope plot was characterized by principal component analysis in SigmaPlot v.13.0. As a result, a characteristic unit vector ($\vec{P}$) was determined for each degradation pathway (see the Supporting Information and Table S1). For a given degradation pathway, the unit vector $\vec{P}$ can also be calculated from ε_{bulk} values determined in laboratory experiments according to the expression:

$$\vec{P} = \frac{1}{\sqrt{(\varepsilon^{Cl}_{bulk})^2 + (\varepsilon^{C}_{bulk})^2 + (\varepsilon^{H}_{bulk})^2}} \times (\varepsilon^{Cl}_{bulk}, \varepsilon^{C}_{bulk}, \varepsilon^{H}_{bulk}) \quad (3)$$

provided that absolute ε_{bulk} values are used (see the Supporting Information). A comparison of $\vec{P}$-vectors determined by principal component analysis with those calculated from eq 3 is available in Table S2.

RESULTS AND DISCUSSION

Hydrogen Isotope Fractionation. *Aerobic Biodegradation Experiments.* The experiments lasted between 12 and 21 h (half-life from ~3.5 to ~6.3 h; see the Supporting Information), and 1,2-DCA transformation above 90% was reached for all replicates. δ^2H values for 1,2-DCA showed a trend toward more positive values during its transformation by C−H bond oxidation (Figure 1a) or hydrolytic dehalogenation (Figure 1b), reflecting an enrichment of 1,2-DCA in the heavy isotope (^2H). This is indicative of a normal isotope effect. The δ^2H values for 1,2-DCA in the controls remained constant throughout the experiments ($\delta^2H_{VSMOW-SLAP}$ = −53 ± 3‰, ± 1σ, n = 6). For *Pseudomonas* sp. strain DCA1 (C−H bond oxidation, Scheme 1a), a very large shift in δ^2H values was observed, with up to $\Delta\delta^2H_{VSMOW-SLAP}$ = +188 ± 10‰ after ~80% degradation (Figure 1a). This resulted in a large ε^H_{bulk} value of −115 ± 18‰ ($AKIE_H$ = 1.6 ± 0.2). Large ε^H_{bulk} values were also observed, for instance, during oxidation of ethylbenzene by *Aromatoleum aromaticum* (ε^H_{bulk} = −111 ± 7‰, $AKIE_H$ = 6.0, for variations of δ^2H within the range $\Delta\delta^2H$ < 100)[41] or methyl *tert*-butyl ether (MTBE) by *Pseudonocardia tetrahydrofuranoxydans* K1 (ε^H_{bulk} = −100 ± 10‰, $AKIE_H$ = 14.2).[42] In comparison to the large ε^H_{bulk} value determined for oxidation of 1,2-DCA, for hydrolytic dehalogenation of 1,2-DCA (C−Cl bond cleavage via S_N2, Scheme 1b) a much lower enrichment in ^2H was measured in experiments with *A.*

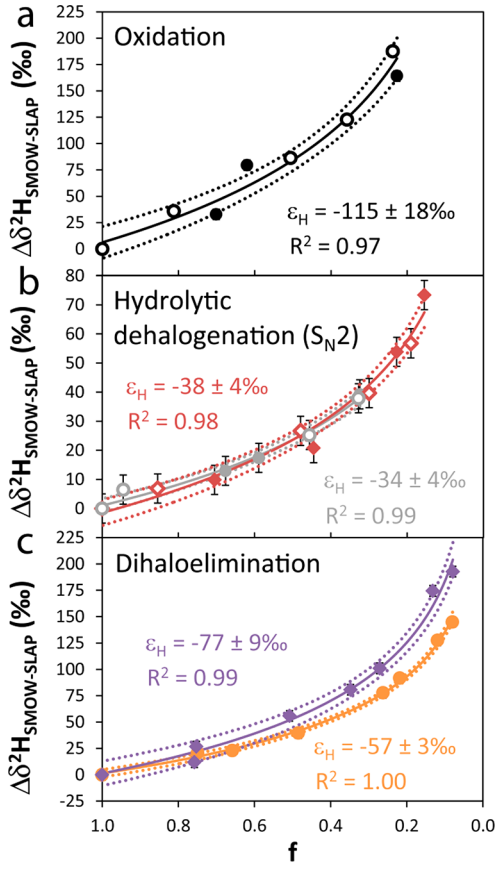


Figure 1. Hydrogen isotopic fractionation of 1,2-DCA during biodegradation by *Pseudomonas* sp. (a), *A. aquaticus* (b, gray circles), *X. autotrophicus* (b, red diamonds) and *Dehalococcoides*- (c, orange circles) and *Dehalogenimonas*-containing cultures (c, violet diamonds); f is the fraction of 1,2-DCA remaining. For the aerobic experiments (a, b), solid and empty symbols represent data from two replicate bottles. The error bars for isotope values in panels a and c are smaller than the symbols. The solid lines are fits in SigmaPlot according to eq 2, and dotted lines show the 95% confidence intervals of the nonlinear regressions.

DOI: 10.1021/acs.est.7b02906
Environ. Sci. Technol. 2017, 51, 10526−10535

aquaticus and *X. autotrophicus* (ε^{H}_{bulk} values of −34 ± 4‰ and −38 ± 4‰, respectively; Figure 1b).

Anaerobic Biodegradation Experiments. The experiments lasted approximately 9 and 40 days (half-life of ∼30 and ∼289 h; see the Supporting Information) for the microcosms with *Dehalococcoides*- and *Dehalogenimonas*-containing cultures, respectively, at which point most all of the initial 1,2-DCA was transformed to ethene via dihaloelimination. VC was detected only in the *Dehalococcoides*-containing microcosms, at concentrations much lower than those of ethene. The maximum accumulation of VC represented less than 6% of the initial 1,2-DCA added. Further information on concentrations of ethene and VC during the degradation of 1,2-DCA is available in a previous study.[14] The concentration pattern of products observed in this previous study[14] indicated that ethene and VC were formed in parallel reaction pathways via dihaloelimination and dehydrohalogenation (Scheme 1), respectively. Hence, products concentrations indicated that only a small fraction of 1,2-DCA was transformed via dehydrohalogenation in the *Dehalococcoides*-containing microcosms.

As observed for the aerobic experiments, enrichment in ^{2}H was obtained during the dihaloelimination of 1,2-DCA by both anaerobic enrichment cultures (ε^{H}_{bulk} values of −57 ± 3‰ and −77 ± 9‰; Figure 1c). However, in contrast to the similar ε^{H}_{bulk} values obtained for hydrolytic dehalogenation by *A. aquaticus* and *X. autotrophicus*, a significantly higher value was determined for dihaloelimination by *Dehalogenimonas* (ε^{H}_{bulk} = −77 ± 9‰) compared to that of *Dehalococcoides*- (ε^{H}_{bulk} = −57 ± 3‰) containing cultures. The $\delta^{2}H$ values for 1,2-DCA in the controls did not change significantly during both experiments (i.e., $\delta^{2}H_{VSMOW-SLAP}$ of −44 ± 1‰ and −50 ± 2‰, ± 1σ, n = 4, respectively). It is also interesting to note that, although 1,2-DCA was purchased from different suppliers in each laboratory (see the Supporting Information), their hydrogen isotopic signatures were relatively similar, varying between $\delta^{2}H_{VSMOW-SLAP}$ values of −44 ± 1‰ and −53 ± 3‰.

Even though clearly distinct ε^{H}_{bulk} values were determined for different aerobic and anaerobic biodegradation pathways for 1,2-DCA during experiments performed in the laboratory, pathway distinction based on isotope fractionation of one element alone is not possible under field conditions. The reason is that changes in substrate concentrations are also related to processes other than transformation (e.g., hydrodynamic dispersion). This prevents the accurate calculation of ε^{H}_{bulk} values and, hence, precludes mechanistic information based on isotope effects. The situation is different if isotope analysis is conducted on two or more elements. The proportion of changes in δ-values of both elements relative to each other (e.g., $\Delta\delta^{2}H/\Delta\delta^{13}C$) is largely unaffected by nondegradative processes.[43,44] Therefore, measurements of isotope fractionation of two or more elements are crucial for investigating contaminant biodegradation pathways in the field.

Multi-element Isotope Approach. Hydrogen δ-values of 1,2-DCA were combined with previously determined carbon and chlorine isotopic data for these experiments[14,24] in dual- ($\delta^{2}H$ versus $\delta^{13}C$ and $\delta^{2}H$ versus $\delta^{37}Cl$) and multi-element ($\delta^{2}H$ versus $\delta^{13}C$ versus $\delta^{37}Cl$) isotope plots. The dual C−H isotope approach resulted in very good linear correlations ($r^{2} \geq$ 0.98, Figure 2), which is consistent with Dorer et al.[41] These authors showed that for variations of $\delta^{2}H$ within the range $\Delta\delta^{2}H$ < 100−200‰, the ε^{H}_{bulk} and Λ_{C-H} values (i.e., $\Lambda_{C-H} = \Delta\delta^{2}H/\Delta\delta^{13}C \approx \varepsilon^{H}_{bulk}/\varepsilon^{C}_{bulk}$) can be evaluated using eq 2 (Figure S1) and $\Delta\delta^{2}H$ against $\Delta\delta^{13}C$ data in a dual-element isotope plot (Figure 2), respectively. A different procedure may be necessary for evaluating the stronger H isotope fractionation that lead to larger shifts in $\delta^{2}H$ because previous studies observed a nonlinear behavior in H isotope ratios in Rayleigh and dual-element isotope plots at a late stage of reaction.[38,41]

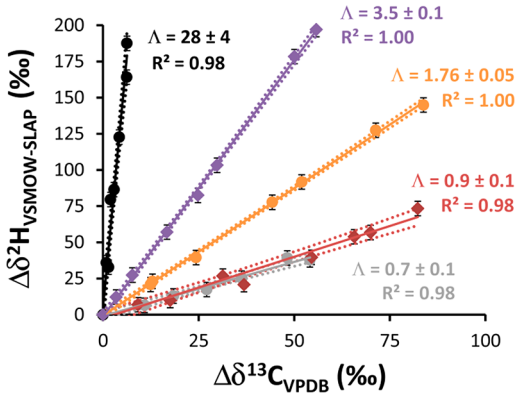


Figure 2. Dual C−H isotope trends during the biodegradation of 1,2-DCA via oxidation by *Pseudomonas* sp. (black circles), hydrolytic dehalogenation by *A. aquaticus* (gray circles) and *X. autotrophicus* (red diamonds), and dihaloelimination by *Dehalococcoides*- (orange circles) and *Dehalogenimonas*-containing cultures (violet diamonds). Dotted lines indicate the 95% confidence intervals of the linear regression. Error bars of $\Delta\delta^{13}C$ values are smaller than the symbols. Λ values (±95% confidence interval) are given by the slope of the linear regressions.

The clearly distinct isotope patterns observed in Figure 2 for all the investigated degradation pathways, with Λ_{C-H} values ranging between 28 ± 4 (oxidation) and 0.8 ± 0.1 (average value for hydrolytic dehalogenation) opens the possibility of a dual C−H isotope approach to identify the different aerobic and anaerobic degradation pathways for 1,2-DCA in the field. Particularly, for aerobic hydrolytic dehalogenation by *X. autotrophicus* and *A. aquaticus* (average Λ_{C-H} value of 0.8 ± 0.1) and reductive dihaloelimination by a *Dehalococcoides*-containing culture (Λ_{C-H} = 1.76 ± 0.05), a larger relative difference in Λ_{C-H} values was obtained (around 50% relative to the higher value) compared to that of their respective Λ_{C-Cl} values (around 10%, Scheme 1). Therefore, the use of a C−H isotope approach enables the improved identification of the two pathways (i.e., hydrolytic dehalogenation and dihaloelimination).

A dual Cl−H isotope approach was also investigated and good linear correlations ($r^{2} \geq$ 0.97; Figure S2) were observed for all the degradation experiments in a dual isotope plot (see the Supporting Information). However, similar Λ_{Cl-H} values were obtained for aerobic hydrolytic dehalogenation by *X. autotrophicus* and *A. aquaticus* (6.5 ± 1.0 and 5.7 ± 0.9, respectively) and dihaloelimination by a *Dehalogenimonas*-containing culture (6.7 ± 0.3), which hampers the differentiation of these two pathways (i.e., hydrolytic dehalogenation and dihaloelimination) using a dual Cl−H isotope approach.

The large differences among all trends obtained for the dual C−H isotope approach (Figure 2) also enables the estimation of the proportion of two competing pathways (e.g., oxidation and hydrolytic dehalogenation reactions under aerobic conditions or dihaloelimination by *Dehalococcoides* and *Dehalogenimonas* populations under anaerobic conditions) based on the resultant slope,[45,46] assuming simultaneous

DOI: 10.1021/acs.est.7b02906
Environ. Sci. Technol. 2017, 51, 10526−10535

activity with a constant ratio between both pathway rates. However, for three or more pathways, unique solutions are not possible. An improved evaluation of up to three different degradation pathways might be possible by combining isotope data of three elements.

The measurement of hydrogen isotope ratios enables the combination of δ^2H with δ^{13}C and δ^{37}Cl data in a multi-element isotope plot (Figure 3). This new approach was

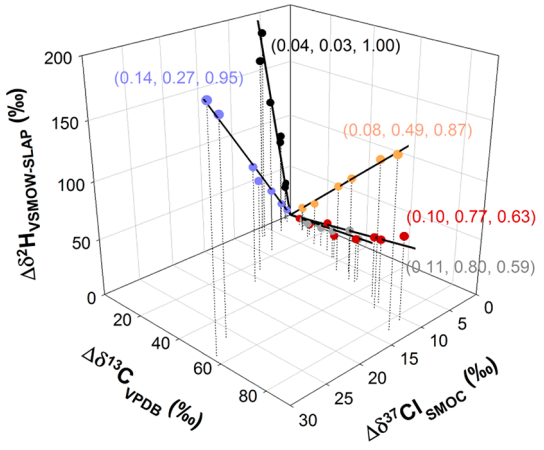


Figure 3. Multi-element isotope patterns during biodegradation of 1,2-DCA via different reaction pathways: oxidation by *Pseudomonas* sp. (black), hydrolytic dehalogenation by *A. aquaticus* (gray) and *X. autotrophicus* (red), and dihaloelimination by *Dehalococcoides*- (orange) and *Dehalogenimonas*-containing cultures (violet). Solid lines are defined by the unit $\vec{P}$-vectors indicated in brackets (Table S1).

investigated to determine whether the multi-element isotope patterns are sufficiently different to potentially distinguish among different aerobic and anaerobic biodegradation pathways in the field. As observed in Figure 3, the strongly different trends further strengthen pathway identification in future biodegradation studies for 1,2-DCA. To identify the degradation pathway of 1,2-DCA using a 3D approach, multi-element isotope data from new field (or laboratory) studies can be characterized by principal component analysis (see the Supporting Information). The obtained $\vec{P}$-vector can then be compared to those reference $\vec{P}$-vectors reported in this study for different reactions (Figure 3) by determining the angle between them (see the Supporting Information and eq S8). In complex sites where different biodegradation pathways may be involved, the occurrence of up to three different pathways might be detected using a 3D approach. For instance, in a site where three potential biodegradation pathways may control the fate of 1,2-DCA (e.g., aerobic biodegradation via oxidation and hydrolytic dehalogenation and anaerobic dihaloelimination by *Dehalogenimonas* populations), data points situated between their respective reference $\vec{P}$-vectors would indicate the effect of all of them. However, unequivocal information is hampered if more than three pathways occur at the site. If there are more than three pathways, additional information (e.g., redox data) is required to constrain pathways.

The distinctly different Λ_{C-H} values in Figure 2 can be rationalized in terms of the corresponding degradation mechanisms. For oxidation of 1,2-DCA, a highly pronounced H isotope fractionation was observed (ε^H_{bulk} = −115 ± 18‰), reflecting a strong primary H isotope effect during oxidative C−H bond cleavage (Scheme 1a). In addition, smaller but non-negligible secondary isotope effects in H atoms located in proximity to the reacting bond (see below) are likely represented in the observable bulk fractionation.[41,47] In contrast, for hydrolytic dehalogenation and dihaloelimination reactions (Schemes 1b−d), smaller ε^H_{bulk} values were determined, ranging from −34 ± 4‰ to −77 ± 9‰ because only secondary H isotope effects are involved during C−Cl bond cleavage. Conversely, a lower ε^C_{bulk} value was obtained for carbon during oxidation (−3.5 ± 0.1‰ for C−H bond cleavage),[24] relative to those determined for hydrolytic dehalogenation and dihaloelimination reactions (from −23 ± 2‰ to −33.0 ± 0.4‰ for C−Cl bond cleavage),[14,24] due to the different mass of its bonding partner.[34] As a result, the largest Λ_{C-H} value was obtained for the C−H oxidation pathway (Figure 2). For the reactions without an initial primary H isotope effect, the different ε^H_{bulk} values for hydrolytic dehalogenation (average value of −36 ± 3‰) and dihaloelimination by *Dehalococcoides* (−57 ± 3‰) and *Dehalogenimonas* (−77 ± 9‰) containing cultures compared with their relatively similar ε^C_{bulk} values (from −23 ± 2‰ to −33.0 ± 0.4‰)[14,24] also resulted in distinctly different Λ_{C-H} values (Figure 2). The variation between ε^H_{bulk} values for hydrolytic dehalogenation and dihaloelimination reactions and also between dihaloelimination by *Dehalococcoides*- and *Dehalogenimonas*-containing cultures is discussed below.

Secondary H Isotope Fractionation and Insight into Anaerobic Dihaloelimination Mechanism. During the transformation of 1,2-DCA by *A. aquaticus* and *X. autotrophicus* by a haloalkane hydrolytic dehalogenase reaction (i.e., nucleophilic substitution S_N2-type,[5,6] Scheme 1b), a single C−Cl bond cleavage occurs in the first reaction step. Therefore, no primary H isotope effect would be expected for this pathway and the measured ε^H_{bulk} values (−34 ± 4‰ and −38 ± 4‰) represent the average secondary H isotope fractionation of all positions, i.e., α-secondary isotope effects in two H atoms located next to the reacting bond and β-secondary isotope effects in two H atoms situated one position away from the reacting bond. The H atoms situated one bond apart from the reacting bond might exhibit a smaller secondary isotope effect (see[33,34] and references herein) compared to those atoms adjacent to the reaction bond. For instance, α-secondary KIE_H = 1.1−1.2 and β-secondary KIE_H = 1.05−1.15 are expected for a nucleophilic substitution S_N1-type involving C−Cl bonds.[34]

Similar to hydrolytic dehalogenation, no primary H isotope effect is expected in the initial transformation of 1,2-DCA by *Dehalococcoides*- and *Dehalogenimonas*-containing cultures because no C−H bond is broken during dihaloelimination of 1,2-DCA to ethene (Scheme 1c). However, much higher secondary ε^H_{bulk} values (−57 ± 3‰ and −77 ± 9‰) were determined for both cultures compared to those obtained for hydrolytic dehalogenation. The large secondary compound average H isotope fractionation values measured for dihaloelimination are ∼50−70% of that measured for C−H oxidation by *Pseudomonas* (−115 ± 18‰), which is remarkable given that no C−H bond is broken in the dihaloelimination reaction.

For enzymatic dihaloelimination of 1,2-DCA, a previous study based on C and Cl isotope fractionation suggested that the difference between ε^C_{bulk} and ε^{Cl}_{bulk} values obtained in experiments with *Dehalococcoides*- and *Dehalogenimonas*-containing cultures could be associated with a different mode of concerted bond cleavage rather than with stepwise versus

DOI: 10.1021/acs.est.7b02906
Environ. Sci. Technol. 2017, 51, 10526−10535

concerted reactions (Scheme 1c, d).[14] Assuming concerted dihaloelimination of 1,2-DCA (Scheme 1c), α-secondary isotope effects may be anticipated for all H atoms. The location of all H atoms next to simultaneously reacting bonds could explain the higher secondary ε^{H}_{bulk} values measured for dihaloelimination compared to hydrolytic dehalogenation, where β-secondary isotope effects are involved in the latter.

α-secondary AKIEs of 1.060 ± 0.003 and 1.08 ± 0.01 were calculated for concerted dihaloelimination of 1,2-DCA by *Dehalococcoides*- and *Dehalogenimonas*-containing cultures, respectively, assuming simultaneous secondary effects without intramolecular competition (see the Supporting Information). These values agree well with the α-secondary KIE_H (i.e., from 0.95 to 1.2) reported for nucleophilic substitution (S_N1- and S_N2-type).[34] The different magnitude of α-secondary $AKIE_H$ during 1,2-DCA transformation by *Dehalococcoides*- and *Dehalogenimonas*-containing cultures might reflect a different interaction mode between reductive dehalogenases and 1,2-DCA (e.g., how leaving groups were stabilized in different enzyme environments) as previously proposed to explain the differences on C and Cl isotope effects (see Palau et al.[14] and references therein). A comparison of the hydrogen AKIEs determined in this study with those obtained using quantum mechanical and molecular mechanical modeling (QM/MM) in future studies can help to elucidate the enzymatic reaction mechanisms in more detail.

The evidence from H isotope ratios obtained in this study is in agreement with concerted dihaloelimination of 1,2-DCA by *Dehalococcoides*- and *Dehalogenimonas*-containing cultures, showing that further insight into enzymatic reductive dechlorination of 1,2-DCA can be obtained from hydrogen isotope fractionation. Such insight cannot be obtained from end product analysis because the same product (i.e., ethene) is formed during the transformation of 1,2-DCA via concerted or stepwise dihaloelimination (Scheme 1c,d). As observed for 1,2-DCA in this study, the different magnitude of secondary H isotope effects might help to differentiate between concerted and stepwise dihaloelimination mechanisms during biodegradation of other chlorinated ethanes of environmental concern, such 1,1,2-trichloroethane or 1,1,2,2-tetrachloroethane, in future studies.

Environmental Significance. In addition to elucidating natural biodegradation processes for 1,2-DCA, multi-element isotope analysis can also be useful for obtaining insight into enhanced remediation processes, such as in situ bioaugmentation.[48−50] This form of enhanced biodegradation typically reduces the time required to reach target remediation goals at contaminated sites. However, its application and evaluation can be difficult at numerous sites impacted by mixtures of chlorinated compounds such as ethenes and ethanes. A recent study by Mayer-Blackwell et al.[51] showed that the reductive dihaloelimination of 1,2-DCA by a *Dehalococcoides mccartyi* consortium was strongly inhibited by *cis*-DCE. These authors also suggested that the presence of a significant 1,2-DCA concentration in groundwater that is co-contaminated with chlorinated ethenes may alter the *Dehalococcoides* population structure away from conditions ideal for complete degradation of VC. In this case, degradation of 1,2-DCA by other ORB such as *Dehalogenimonas* could eventually eliminate its inhibitory effect on VC degradation by *Dehalococcoides* populations in the field. Therefore, knowledge of the fate of 1,2-DCA at sites co-contaminated with chlorinated ethenes is crucial for site remediation. However, the assessment of 1,2-DCA transformation by *Dehalococcoides* or *Dehalogenimonas* populations in groundwater co-contaminated with chlorinated ethenes is particularly challenging because the same products (i.e., ethene and VC) are also obtained during biodegradation of chlorinated ethenes. The results of this study show that dihaloelimination of 1,2-DCA by ORB such as *Dehalococcoides* or *Dehalogenimonas* could be identified using a dual- (δ^2H versus δ^{13}C) and a multi-element (δ^2H vs δ^{13}C vs δ^{37}Cl) isotope approach, illustrating the potential of H isotope analysis in combination with C and Cl isotope data to investigate transformation pathways for 1,2-DCA in the field. In addition to the ORBs investigated in this study, other anaerobic bacteria are also able to reductively dechlorinate 1,2-DCA. Therefore, the multi-element isotope patterns determined for the *Dehalococcoides*- and *Dehalogenimonas*-containing enrichment cultures should be compared to the patterns obtained using other types of microbes, such *Dehalobacter*[8] or *Desulfitobacterium*,[52] in future studies.

Identification of the 1,2-DCA degradation pathway in the field can help constrain the range of ε_{bulk} values used to estimate contaminant degradation extent using the Rayleigh equation,[53,54] which is one of the main applications of CSIA to field studies. The new hydrogen isotope fractionation values for 1,2-DCA determined in this study, under both oxic and anoxic conditions, opens the possibility for using H−CSIA to quantify degradation at sites polluted by 1,2-DCA. In addition, the ε^{H}_{bulk} values for the different reaction pathways determined in this study are larger than their respective ε^{C}_{bulk} [13,14,24,35,55−58] and ε^{Cl}_{bulk} [14,24] values, indicating that hydrogen isotopic fractionation can be a more sensitive indicator than carbon and chlorine isotopic fractionation. This can be particularly important for obtaining a better assessment of microbial oxidation of 1,2-DCA in the field (ε^{H}_{bulk} = −115 ± 18‰ compared to the ε^{C}_{bulk} average value of −3.8 ± 0.8‰, ±1σ, n = 6,[24,57] and the ε^{Cl}_{bulk} value of −3.8 ± 0.2).[24]

Groundwater contaminant plumes are dynamic and highly heterogeneous systems subject to temporal and spatial geochemical variations that control biodegradation processes in an aquifer.[59] At contaminated sites, multi-isotope analysis in combination with appropriately high-resolution sampling could be used to investigate the distribution of 1,2-DCA biodegradation processes associated with steep redox gradients, from oxic to anoxic environments (e.g., as often occurs with depth), which has rarely been considered in contaminant biodegradation studies. Understanding active degradation processes in the field is essential for evaluating natural attenuation and predicting how far a groundwater plume of 1,2-DCA might migrate. In this case, it is important to know if aerobic or anaerobic biodegradation occurs, especially because aerobic degradation rates for 1,2-DCA are likely controlled by the availability of oxygen. Hence, the conceptual model and, accordingly, the mathematical model used to predict plume behavior would be quite different. It also has implications for enhancing one remediation approach over another, e.g., adding more oxygen or adding electron donor, depending on the identified biodegradation pathway. Based on the results of this study, multi-element isotope patterns are expected (i) to identify the active transformation pathway for 1,2-DCA and (ii) to detect changes in biodegradation conditions (i.e., aerobic versus anaerobic biodegradation), allowing for better characterization of the degradation processes in the field.

DOI: 10.1021/acs.est.7b02906
Environ. Sci. Technol. 2017, 51, 10526−10535

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b02906.

Further information about chemicals, analytical methods, reaction kinetics, correction of the substrate remaining fraction, Rayleigh isotope plot, calculation of H-AKIEs, dual Cl−H isotope plot, and characterization of multi-element (Cl, C, and H) isotope trends. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jordi.palau@ub.edu.

ORCID

Jordi Palau: 0000-0001-9492-7306

Monica Rosell: 0000-0003-1563-8595

David L. Freedman: 0000-0001-6778-3706

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thoroughly thank the four anonymous reviewers and the editor for their helpful comments on the manuscript. We also thank Dr. Raimon Tolosana-Delgado for his comments on data analysis. J.P. was supported by the University of Neuchâtel via direct university funding. E.M.-U. is supported by the Spanish Ministry of Economy and Competitiveness and FEDER (project no. CTM2013-48545-C2-1-R) and the Xarxa de Referència en Biotecnologia de la Generalitat de Catalunya. The MAG group is supported by Spanish Government REMEDIATION project (CGL2014-57215-C4-1-R) and the Catalan Government project no. 2014SGR-1456. S.H.M acknowledges support from the Ministry of Education Malaysia (SLAI-UMP Scholarship) for a predoctoral fellowship, and M.R. acknowledges a Ramón y Cajal contract (RYC-2012-11920).

■ REFERENCES

(1) ATSDR. Toxicological Profile for 1,2-Dichloroethane. http://www.atsdr.cdc.gov/toxfaqs/tfacts38.pdf (accessed July 17, 2017).

(2) U.S. EPA. Releases of Chemicals in the 2015 TRI National Analysis. https://www.epa.gov/trinationalanalysis/releases-chemicals-2015-tri-national-analysis (accessed March 14, 2017).

(3) U.S. EPA. Priority pollutants. http://water.epa.gov/scitech/methods/cwa/pollutants.cfm (accessed Jan 7, 2015).

(4) Hage, J. C.; Hartmans, S. Monooxygenase-mediated 1,2-dichloroethane degradation by Pseudomonas sp. strain DCA1. *Appl. Environ. Microbiol.* **1999**, *65* (6), 2466−70.

(5) 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.

(6) 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.

(7) 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.

(8) 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.

(9) 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.

(10) 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.

(11) 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.

(12) 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.

(13) 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.

(14) Palau, J.; Yu, R.; Hatijah Mortan, S.; Shouakar-Stash, O.; Rosell, M.; Freedman, D. L.; Sbarbati, C.; Fiorenza, S.; Aravena, R.; Marco-Urrea, E.; Elsner, M.; Soler, A.; Hunkeler, D. Distinct Dual C-Cl Isotope Fractionation Patterns during Anaerobic Biodegradation of 1,2-Dichloroethane: Potential To Characterize Microbial Degradation in the Field. *Environ. Sci. Technol.* **2017**, *51* (5), 2685−2694.

(15) Gossett, J. M. Sustained aerobic oxidation of vinyl chloride at low oxygen concentrations. *Environ. Sci. Technol.* **2010**, *44* (4), 1405−11.

(16) 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.

(17) Dinglasan-Panlilio, M. J.; Dworatzek, S.; Mabury, S.; Edwards, E. Microbial oxidation of 1,2-dichloroethane under anoxic conditions with nitrate as electron acceptor in mixed and pure cultures. *FEMS Microbiol. Ecol.* **2006**, *56* (3), 355−64.

(18) 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.

(19) 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.

(20) 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.

(21) Abe, Y.; Aravena, R.; Zopfi, J.; Shouakar-Stash, O.; Cox, E.; Roberts, J. D.; Hunkeler, D. Carbon and Chlorine Isotope Fractionation during Aerobic Oxidation and Reductive Dechlorination of Vinyl Chloride and cis-1,2-Dichloroethene. *Environ. Sci. Technol.* **2009**, *43* (1), 101−107.

(22) Cretnik, S.; Thoreson, K. A.; Bernstein, A.; Ebert, K.; Buchner, D.; Laskov, C.; Haderlein, S.; Shouakar-Stash, O.; Kliegman, S.; McNeill, K.; Elsner, M. Reductive Dechlorination of TCE by Chemical Model Systems in Comparison to Dehalogenating Bacteria: Insights from Dual Element Isotope Analysis (13C/12C, 37Cl/35Cl). *Environ. Sci. Technol.* **2013**, *47* (13), 6855−6863.

(23) 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.

(24) 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

DOI: 10.1021/acs.est.7b02906
Environ. Sci. Technol. 2017, 51, 10526−10535

Identification and Reveals Surprising C-Cl Bond Involvement in Microbial Oxidation. *Environ. Sci. Technol.* **2014**, *48* (16), 9430−7.
(25) Zwank, L. *Assessment of the Fate of Organic Groundwater Contaminants Using Their Isotopic Signatures*; Swiss Federal Institute of Technology (ETH): Zurich, Switzerland, 2004.
(26) Shouakar-Stash, O.; Drimmie, R. J. Online methodology for determining compound-specific hydrogen stable isotope ratios of trichloroethene and 1,2-cis-dichloroethene by continuous-flow isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **2013**, *27* (12), 1335−1344.
(27) Renpenning, J.; Kummel, S.; Hitzfeld, K. L.; Schimmelmann, A.; Gehre, M. Compound-specific hydrogen isotope analysis of heteroatom-bearing compounds via gas chromatography-chromium-based high-temperature conversion (Cr/HTC)-isotope ratio mass spectrometry. *Anal. Chem.* **2015**, *87* (18), 9443−50.
(28) Kuder, T.; Philp, P. Demonstration of compound-specific isotope analysis of hydrogen isotope ratios in chlorinated ethenes. *Environ. Sci. Technol.* **2013**, *47* (3), 1461−7.
(29) Renpenning, J.; Schimmelmann, A.; Gehre, M. Compound-specific hydrogen isotope analysis of fluorine-, chlorine-, bromine- and iodine-bearing organics using gas chromatography-chromium-based high-temperature conversion (Cr/HTC) isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **2017**, *31* (13), 1095−1102.
(30) Audi-Miro, C.; Cretnik, S.; Torrento, C.; Rosell, M.; Shouakar-Stash, O.; Otero, N.; Palau, J.; Elsner, M.; Soler, A. C, Cl and H compound-specific isotope analysis to assess natural versus Fe(0) barrier-induced degradation of chlorinated ethenes at a contaminated site. *J. Hazard. Mater.* **2015**, *299*, 747−54.
(31) Ivdra, N.; Fischer, A.; Herrero-Martin, S.; Giunta, T.; Bonifacie, M.; Richnow, H. H. Carbon, Hydrogen and Chlorine Stable Isotope Fingerprinting for Forensic Investigations of Hexachlorocyclohexanes. *Environ. Sci. Technol.* **2017**, *51* (1), 446−454.
(32) Kuder, T.; van Breukelen, B. M.; Vanderford, M.; Philp, P. 3D-CSIA: carbon, chlorine, and hydrogen isotope fractionation in transformation of TCE to ethene by a Dehalococcoides culture. *Environ. Sci. Technol.* **2013**, *47* (17), 9668−77.
(33) 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.
(34) 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.
(35) 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.
(36) 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.
(37) Elsner, M.; Couloume, G. L.; Lollar, B. S. Freezing to preserve groundwater samples and improve headspace quantification limits of water-soluble organic contaminants for carbon isotope analysis. *Anal. Chem.* **2006**, *78* (21), 7528−7534.
(38) Wijker, R. S.; Adamczyk, P.; Bolotin, J.; Paneth, P.; Hofstetter, T. B. Isotopic analysis of oxidative pollutant degradation pathways exhibiting large H isotope fractionation. *Environ. Sci. Technol.* **2013**, *47* (23), 13459−68.
(39) Buchner, D.; Jin, B.; Ebert, K.; Rolle, M.; Elsner, M.; Haderlein, S. B. Experimental Determination of Isotope Enrichment Factors - Bias from Mass Removal by Repetitive Sampling. *Environ. Sci. Technol.* **2017**, *51* (3), 1527−1536.
(40) 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.
(41) Dorer, C.; Hohener, P.; Hedwig, N.; Richnow, H. H.; Vogt, C. Rayleigh-based concept to tackle strong hydrogen fractionation in dual isotope analysis-the example of ethylbenzene degradation by Aromatoleum aromaticum. *Environ. Sci. Technol.* **2014**, *48* (10), 5788−97.
(42) McKelvie, J. R.; Hyman, M. R.; Elsner, M.; Smith, C.; Aslett, D. M.; Lacrampe-Couloume, G.; Lollar, B. S. Isotopic fractionation of methyl tert-butyl ether suggests different initial reaction mechanisms during aerobic biodegradation. *Environ. Sci. Technol.* **2009**, *43* (8), 2793−2799.
(43) 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.
(44) 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.
(45) Centler, F.; Hesse, F.; Thullner, M. Estimating pathway-specific contributions to biodegradation in aquifers based on dual isotope analysis: Theoretical analysis and reactive transport simulations. *J. Contam. Hydrol.* **2013**, *152*, 97−116.
(46) van Breukelen, B. M. Extending the Rayleigh equation to allow competing isotope fractionating pathways to improve quantification of biodegradation. *Environ. Sci. Technol.* **2007**, *41* (11), 4004−10.
(47) Elsner, M.; Mckelvie, J.; Couloume, G. L.; Lollar, B. S. Insight into methyl tert-butyl ether (MTBE) stable isotope Fractionation from abiotic reference experiments. *Environ. Sci. Technol.* **2007**, *41* (16), 5693−5700.
(48) Ellis, D. E.; Lutz, E. J.; Odom, J. M.; Buchanan, R. J.; Bartlett, C. L.; Lee, M. D.; Harkness, M. R.; DeWeerd, K. A. Bioaugmentation for Accelerated In Situ Anaerobic Bioremediation. *Environ. Sci. Technol.* **2000**, *34* (11), 2254−2260.
(49) Major, D. W.; McMaster, M. L.; Cox, E. E.; Edwards, E. A.; Dworatzek, S. M.; Hendrickson, E. R.; Starr, M. G.; Payne, J. A.; Buonamici, L. W. Field demonstration of successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene. *Environ. Sci. Technol.* **2002**, *36* (23), 5106−16.
(50) Maes, A.; van Raemdonck, H.; Smith, K.; Ossieur, W.; Lebbe, L.; Verstraete, W. Transport and activity of Desulfitobacterium dichloroeliminans strain DCA1 during bioaugmentation of 1,2-DCA-contaminated groundwater. *Environ. Sci. Technol.* **2006**, *40* (17), 5544−5552.
(51) Mayer-Blackwell, K.; Fincker, M.; Molenda, O.; Callahan, B.; Sewell, H.; Holmes, S.; Edwards, E. A.; Spormann, A. M. 1,2-Dichloroethane Exposure Alters the Population Structure, Metabolism, and Kinetics of a Trichloroethene-Dechlorinating Dehalococcoides mccartyi Consortium. *Environ. Sci. Technol.* **2016**, *50* (22), 12187−12196.
(52) 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.
(53) 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.
(54) Aelion, C. M.; Hohëner, P.; Hunkeler, D.; Aravena, R. *Environmental Isotopes in Biodegradation and Bioremediation*; CRC Press: Boca Raton, FL, 2010; pp *xiv*−450.
(55) 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.
(56) 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 hydro-

DOI: 10.1021/acs.est.7b02906
Environ. Sci. Technol. 2017, 51, 10526−10535

carbons in a biostimulation study: added value via stable carbon isotope analysis. *J. Contam. Hydrol.* **2007**, *94* (3−4), 249−60.
(57) 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.
(58) 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.
(59) 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.

DOI: 10.1021/acs.est.7b02906
Environ. Sci. Technol. 2017, 51, 10526−10535