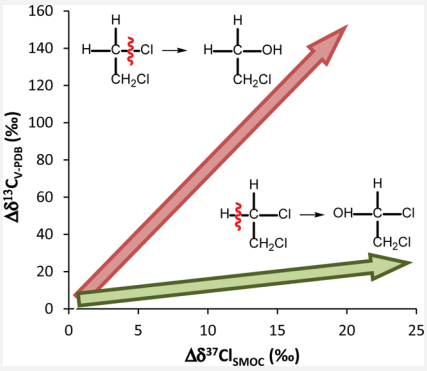
Published in Environmental science & technology 48, 16, 9430-9437, 2014
which should be used for any reference to this work

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

Jordi Palau,*[,†] Stefan Cretnik,[‡] Orfan Shouakar-Stash,[§] Martina Höche,[‡] Martin Elsner,[‡] and Daniel Hunkeler[†]

[†]Centre for Hydrogeology and Geothermics, University of Neuchâtel, 2000 Neuchâtel, Switzerland
[‡]Helmholtz Zentrum München, German Research Center for Environmental Health, D-85764 Neuherberg, Germany
[§]Department of Earth and Environmental Sciences, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

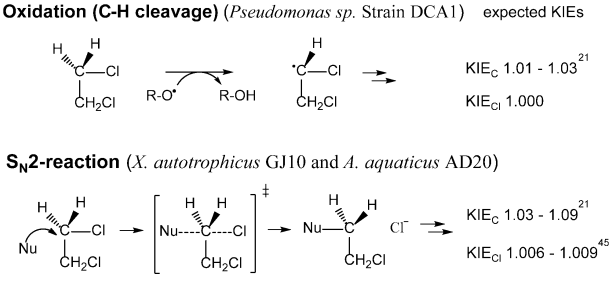
ABSTRACT: This study investigates dual element isotope fractionation during aerobic biodegradation of 1,2-dichloroethane (1,2-DCA) via oxidative cleavage of a C−H bond (*Pseudomonas* sp. strain DCA1) versus C−Cl bond cleavage by S_N2 reaction (*Xanthobacter autotrophicus* GJ10 and *Ancylobacter aquaticus* AD20). Compound-specific chlorine isotope analysis of 1,2-DCA was performed for the first time, and isotope fractionation (ε^{Cl}_{bulk}) was determined by measurements of the same samples in three different laboratories using two gas chromatography−isotope ratio mass spectrometry systems and one gas chromatography−quadrupole mass spectrometry system. Strongly pathway-dependent slopes ($\Delta\delta^{13}C/\Delta\delta^{37}Cl$), 0.78 ± 0.03 (oxidation) and 7.7 ± 0.2 (S_N2), delineate the potential of the dual isotope approach to identify 1,2-DCA degradation pathways in the field. In contrast to different ε^{C}_{bulk} values [−3.5 ± 0.1‰ (oxidation) and −31.9 ± 0.7 and −32.0 ± 0.9‰ (S_N2)], the obtained ε^{Cl}_{bulk} values were surprisingly similar for the two pathways: −3.8 ± 0.2‰ (oxidation) and −4.2 ± 0.1 and −4.4 ± 0.2‰ (S_N2). Apparent kinetic isotope effects (AKIEs) of 1.0070 ± 0.0002 (^{13}C-AKIE, oxidation), 1.068 ± 0.001 (^{13}C-AKIE, S_N2), and 1.0087 ± 0.0002 (^{37}Cl-AKIE, S_N2) fell within expected ranges. In contrast, an unexpectedly large secondary ^{37}Cl-AKIE of 1.0038 ± 0.0002 reveals a hitherto unrecognized involvement of C−Cl bonds in microbial C−H bond oxidation. Our two-dimensional isotope fractionation patterns allow for the first time reliable 1,2-DCA degradation pathway identification in the field, which unlocks the full potential of isotope applications for this important groundwater contaminant.



INTRODUCTION

Chlorinated ethanes are among the most widespread contaminants in groundwater,[1] and 1,2-dichloroethane (1,2-DCA) has been found in 36% of 1585 National Priorities List sites identified by the U.S. Environmental Protection Agency.[2] The presence of 1,2-DCA, an intermediate in the production of plastics, in groundwater is mainly a consequence of industrial activity.[2] A number of laboratory[3−9] and field[10,11] studies showed 1,2-DCA biodegradation under aerobic[3−6] and anaerobic[4,7−11] conditions via different reaction pathways.[9] Under aerobic conditions, 1,2-DCA can be degraded either via nucleophilic substitution (S_N2)[5,6] or via oxidative cleavage of a C−H bond[3] catalyzed by a hydrolytic dehalogenase or monooxygenase enzyme, respectively (Scheme 1). Initial products of both reactions, 2-chloroethanol (S_N2 reaction) and 1,2-dichloroethanol (oxidation), are further degraded to ubiquitous end products, which hampers a direct identification of degradation pathways from metabolite analysis. Alternative approaches to detecting and identifying 1,2-DCA transformation pathways in the subsurface are therefore warranted.

Scheme 1



This is crucial information in environmental field studies for assessing 1,2-DCA natural attenuation.

Compound-specific isotope analysis (CSIA) is an innovative tool for investigating degradation pathways of organic

contaminants.[12−14] Isotope ratios of individual compounds, measured either by gas chromatography and isotope ratio mass spectrometry (GC−IRMS) or gas chromatography and quadrupole mass spectrometry (GC−qMS), are reported using the delta notation:

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

where R is the isotope ratio of heavy (^{h}E) and light (^{l}E) isotopes of element E (e.g., $^{13}C/^{12}C$ and $^{37}Cl/^{35}Cl$). The isotope fractionation (ε) expresses by how much $^{h}E/^{l}E$ is smaller (negative values) or larger (positive values) in the average of freshly formed products compared to that in the substrate from which they are produced. Transformation-induced isotope fractionation is generally larger than the one related to phase transfer processes such as sorption or volatilization.[15] Bulk (i.e., compound-average) ε values can be calculated using a modified form of the Rayleigh distillation equation:

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

where R_t and R_0 are the current and initial isotope ratios, respectively, and f is the remaining fraction of the compound.

Previous laboratory experiments[16] showed that for 1,2-DCA different carbon ε_{bulk} values of −29.2 and −3.9‰ reflected different reaction pathways: hydrolytic dehalogenation (C−Cl bond cleavage via S_N2) and oxidation (C−H bond cleavage), respectively (Scheme 1). Knowledge of *in situ* contaminant biodegradation reactions is essential for evaluating the fate and long-term impact of 1,2-DCA in groundwater. For aerobic biodegradation of 1,2-DCA, isotope data are particularly important as no characteristic products accumulate. However, while isotope fractionation of one element alone can distinguish pathways in laboratory experiments (where mass balances can be established and ε_{bulk} values determined), this is not possible under field conditions. Here, evidence of a second element and a dual isotope approach is necessary to distinguish 1,2-DCA degradation pathways.[17] As observed experimentally,[18,19] for a given compound, combined isotope analysis of two elements (e.g., $\delta^{13}C$ vs $\delta^{37}Cl$) during the course of a reaction generally yields a linear trend in a dual element isotope plot with a slope characteristic of the reaction mechanism. The reason is that the dual element isotope slope ($\Lambda = \Delta\delta^{13}C/\Delta\delta^{37}Cl$, where $\Delta\delta^{13}C$ and $\Delta\delta^{37}Cl$ are changes in isotope ratios during degradation) reflects isotope fractionation of both elements.[12] Therefore, different slopes are expected from distinct reaction pathways involving different bonds with different elements.

Currently, there is growing interest in dual element isotope analysis for improved differentiation of transformation mechanisms, and several authors pointed out that complementary mechanistic insight for 1,2-DCA aerobic biodegradation reactions could be achieved by the additional analysis of chlorine[12] and/or hydrogen[16,20] isotope ratios. The reason is that isotope fractionation can be traced back to underlying kinetic isotope effects, which are highly reaction-specific.[21] During enzymatic degradation, molecules containing the light isotope at the reactive site (e.g., ^{35}Cl) typically exhibit a reaction rate (e.g., ^{35}k) higher than the rate of those with a heavy isotope (e.g., ^{37}Cl), resulting in a kinetic isotope effect (KIE) of $^{35}k/^{37}k$.[22] When a C−Cl bond is broken, a (primary) chlorine leaving group KIE would be expected, whereas in the oxidation reaction, chlorine atoms sit next to the reacting bond so that only a secondary KIE would be expected (Scheme 1). Secondary isotope effects at positions next to the reacting bond are generally much smaller than primary isotope effects.[12]

Until recently, Cl-CSIA of chlorinated aliphatic compounds was not feasible, however, because a direct method that would produce a suitable Cl-containing measurement gas inside a chromatographic separation gas was lacking. However, new analytical methods were developed on the basis of the measurement of selected isotopologue ions or isotopologue ion fragments in unconverted analyte molecules using both continuous flow GC−IRMS[23] and GC−qMS.[24−26] In addition, a theoretical framework provided the theoretical justification for such evaluation of isotope fractionation from ion current ratios of molecular and fragment ion multiplets.[27] A recent interlaboratory study took the next step and demonstrated that comparable $\delta^{37}Cl$ values were obtained upon analysis of a set of pure trichlorethene (TCE) standards on eight different instruments.[28] Because the technology is so new, however, a comparative study that shows that comparable ε_{Cl} values are obtained when analyzing degradation samples on different instruments and in different laboratories would also be desired. No such study has yet been conducted. Most notably, Cl-CSIA studies have so far been applied to only few compounds, because two isotopically distinct compound-specific standards are necessary for every new substance.[28] This has restricted applications primarily to chlorinated ethylenes,[18,19,29−34] so that, to the best of our knowledge, dual element isotope data are currently nonexistent for chlorinated ethanes.

This study, therefore, aimed (i) to establish for the first time dual element (C and Cl) isotope analysis of the chlorinated ethane 1,2-DCA, (ii) to perform the Cl isotopic analysis in three different laboratories (i.e., Waterloo, München, and Neuchâtel), using two different GC−IRMS systems and one GC−qMS system, to investigate the consistency of ε_{Cl} values obtained with different instruments and analytical methods, and (iii) to investigate carbon and chlorine isotope fractionation during aerobic biodegradation of 1,2-DCA with three pure strains to determine whether the dual isotope slopes are sufficiently different to potentially distinguish between hydrolytic dehalogenation (S_N2) and oxidation (C−H bond cleavage) in the field.

MATERIALS AND METHODS

Preparation of Pure Cultures. Three pure strains with known initial biotransformation mechanisms were used for the batch experiments: *Pseudomonas* sp. strain DCA1 (oxidation) and *Xanthobacter autotrophicus* GJ10 and *Ancylobacter aquaticus* AD20 (S_N2 reaction). *X. autotrophicus* GJ10 (DSMZ 3874) and *A. aquaticus* AD20 (DSMZ 9000) were purchased (DSMZ, Braunschweig, Germany), and *Pseudomonas* sp. strain DCA1 was kindly provided by E. Edwards (Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON). The growth medium was prepared as described by Hunkeler and Aravena.[35]

Cultures and experiments 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). Cultures were amended with 1,2-DCA (Fluka, ≥99.5% pure) and incubated in the dark at room temperature while being continuous shaken (100 rpm). Headspace 1,2-DCA concentrations were monitored throughout the incubation

period. Before the experiments were started, the cultures were transferred three times. Each subculture was spiked with pure 1,2-DCA four times before a 15 mL aliquot was transferred to 170 mL of autoclaved fresh medium. The spike volume was 9 μL of pure 1,2-DCA for the first and second subcultures and 22.5 μL for the third, leading to aqueous phase concentrations of 0.6 and 1.5 mM, respectively.

Experiment Sampling. All experiments were conducted in triplicate. Experiments and controls were amended with 22.5 μL of pure 1,2-DCA, corresponding to the chlorine isotopic working standard ($\delta^{37}Cl_0$-CHYN2 = 0.8 ± 0.1‰), to produce an initial aqueous concentration of 1.5 mM. Bottles were shaken upside down to prevent leakage of the gas phase through the valve. After being shaken for 1 h, samples representing the initial concentration were collected. For concentration and isotopic analysis, aqueous samples (1.5 mL) were taken at selected time points and preserved frozen[36] in 2 mL vials with NaN_3 (1 g/L). Two abiotic control bottles were prepared with 185 mL of autoclaved mineral medium, and samples were collected and preserved as described for the experiments. For each culture experiment, triplicate samples were shipped frozen to the University of Waterloo and to the Helmholtz Zentrum München for chlorine isotope measurements.

Isotopic and Concentration Analysis. Five pure 1,2-DCA isotopic working standards, one for carbon and four for chlorine, were used for instrument monitoring and external calibration of sample raw δ^{37}Cl values to the international Standard Mean Ocean Chloride (SMOC) scale. The isotopic signature of the carbon standard ($\delta^{13}C_{V\text{-}PDB}$ = −29.47 ± 0.05‰) was determined beforehand using an elemental analyzer coupled to an IRMS system. With regard to the chlorine standards, CHYN1 and CHYN2 ($\delta^{37}Cl_{SMOC}$ values of 6.30 ± 0.06 and 0.84 ± 0.14‰, respectively) were characterized relative to SMOC in München by IRMS after conversion of 1,2-DCA to methyl chloride according to the method of Holt et al.[37] IT2-3001 and IT2-3002 ($\delta^{37}Cl_{SMOC}$ values of 0.83 ± 0.09 and −0.19 ± 0.12‰, respectively) were calibrated against the CHYN standards using a GC−IRMS system in Waterloo and a GC−qMS system in Neuchâtel, respectively.

A detailed description of analytical methods is available in the Supporting Information. Carbon isotope ratios were measured by GC−IRMS, and the precision based on the working standard δ^{13}C value reproducibility was 0.5‰ (1σ). Chlorine isotopic analysis was performed using the following instruments: (1) in Waterloo, a model 6890 GC instrument (Agilent, Santa Clara, CA) coupled to a continuous flow IsoPrime IRMS instrument (Micromass, Manchester, U.K.; currently Isoprime Ltd.); (2) in München, a TRACE GC instrument (Thermo Fisher Scientific, Milan, Italy) directly coupled to a Finnigan MAT 253 IRMS instrument (Thermo Fisher Scientific, Bremen, Germany); and (3) in Neuchâtel, a model 7890A GC instrument coupled to a model 5975C quadrupole MS instrument (Agilent). The instrument used in Neuchâtel will be termed the qMS instrument, and those used in Waterloo and München will be termed IRMS-1 and IRMS-2 instruments, respectively.

For analyzing 1,2-DCA, two ions of the molecular group (m/z 100 and 102) were measured with the IRMS-2 instrument, whereas the two most abundant fragment ions (m/z 62 and 64) were used for the IRMS-1 instrument and the qMS instrument. For 1,2-DCA, the intensities of the most abundant fragment ion peaks are much higher than those of the parent ion peaks. Both ion couples (m/z 100 and 102 and m/z 62 and 64) correspond to isotopologue pairs ($[^{37}\mathbf{Cl}_2{}^{12}C_2{}^{1}H_4]^+$ and $[^{37}\mathbf{Cl}^{35}Cl^{12}C_2{}^{1}H_4]^+$, respectively, and $[^{37}\mathbf{Cl}^{12}C_2{}^{1}H_3]^+$ and $[^{35}\mathbf{Cl}^{12}C_2{}^{1}H_3]^+$, respectively) that differ by one heavy chlorine isotope. The isotope ratio (R) can be obtained from the ratio of these isotopologues according to eq 3[27]

$$R = \frac{^{37}Cl}{^{35}Cl} = \frac{^{37}p}{^{35}p} = \frac{k}{n-k+1} \times \frac{^{37}Cl_{(k)}{}^{35}Cl_{(n-k)}}{^{37}Cl_{(k-1)}{}^{35}Cl_{(n-k+1)}} = 2 \times \frac{^{102}I}{^{100}I} = \frac{^{64}I}{^{62}I} \quad (3)$$

where ^{37}p and ^{35}p are the probabilities of encountering ^{37}Cl and ^{35}Cl, respectively, n is the number of Cl atoms, k is the number of ^{37}Cl isotopes, $^{37}Cl_{(k)}{}^{35}Cl_{(n-k)}$ and $^{37}Cl_{(k-1)}{}^{35}Cl_{(n-k+1)}$ represent the isotopologues containing k and $k-1$ heavy isotopes, respectively, and I indicates the peak intensity of each ion.

For the qMS instrument, isotope ratios were calculated using eq 3 and raw δ^{37}Cl values were determined by referencing versus an external 1,2-DCA working standard according to eq 1. In this case, the standard was dissolved in water and measured like the samples in the same sequence.[26] In the IRMS-1 and IRMS-2 instruments, raw δ^{37}Cl values were determined by automatic evaluation of sample's target ion peaks against the ion peaks of the 1,2-DCA monitoring gas, which was introduced by a dual inlet system during each sample run providing an anchor between sample measurements.[28] Subsequently, a two-point linear calibration of raw δ^{37}Cl values to SMOC scale was performed in each laboratory using two external working standards, i.e., IT2(3001 and 3002) for the IRMS-1 instrument and CHYN(1 and 2) for the IRMS-2 and qMS instruments.[28] The analysis schemes applied in each laboratory are available as Supporting Information. For the measurements with the qMS instrument, samples and standards were diluted to a similar concentration and each of them was measured 10 times, leading to a precision (1σ) on the analysis of standards of ±0.3‰. For the IRMS-1 and IRMS-2 instruments, the precision on the analysis of standards was ±0.1‰ (1σ). The δ^{37}Cl of controls remained constant (0.7 ± 0.2‰; n = 12) at $\delta^{37}Cl_0$ within the analytical uncertainty throughout the experiment.

Concentrations of 1,2-DCA were measured by headspace analysis using a TRACE GC-DSQII MS instrument (Thermo Fisher Scientific, Waltham, MA) in single-ion mode (m/z 51, 62, 64, 98, and 100). Concentrations were determined using a five-point calibration curve. The estimated total relative error on analysis of external standards interspersed along the sequence was ±4%. Concentrations were corrected for 1,2-DCA volatilization to the bottle headspace using Henry's law and Henry coefficients.[38] An aqueous phase 1,2-DCA concentration decrease of <5%, caused by a change in the headspace to solution ratio during the experiment, was estimated. The average concentration of controls remained constant throughout the experiments (1.55 ± 0.03 mM, ±1σ; n = 12), indicating that losses of the compound through the valves and caps during the shaking of bottles and preservation of samples were insignificant.

Calculation of Apparent Kinetic Isotope Effects (AKIEs). Kinetic isotope effects are position-specific, whereas ε_{bulk} values are calculated from compound-average isotope data.

Table 1. Chlorine Isotopic Fractionation (ε^{Cl}_{bulk}) Values from Pure Cultures and *p* Values for a Paired *t* Test between the Instruments Used

			IRMS-1		IRMS-2		qMS		
	n^a	r^{2b}	average	variance[c]	average	variance[c]	average	variance[c]	*p* values[d]
A. aquaticus	3	≥0.96	−4.37	0.01	−4.32	0.02	−4.63	0.04	0.923/0.390/0.670
X. autotrophicus	3	≥0.98	−4.343	0.005	−3.960	0.007	−4.39	0.01	0.034/0.829/0.111
Pseudomonas sp.	3	≥0.95	−4.08	0.03	−3.64	0.03	−3.650	0.007	0.085/0.249/0.946

[a]Number of replicates. [b]Correlation coefficient of least-squares regression according to eq 2. [c]The variance among triplicate experiments was determined from the variance of regression for each ε_i as $s_\varepsilon^2 = (s_{\varepsilon 1}^2 + s_{\varepsilon 2}^2 + s_{\varepsilon 3}^2)/9$.[39] [d]IRMS-1 vs IRMS-2/IRMS-1 vs qMS/IRMS-2 vs qMS.

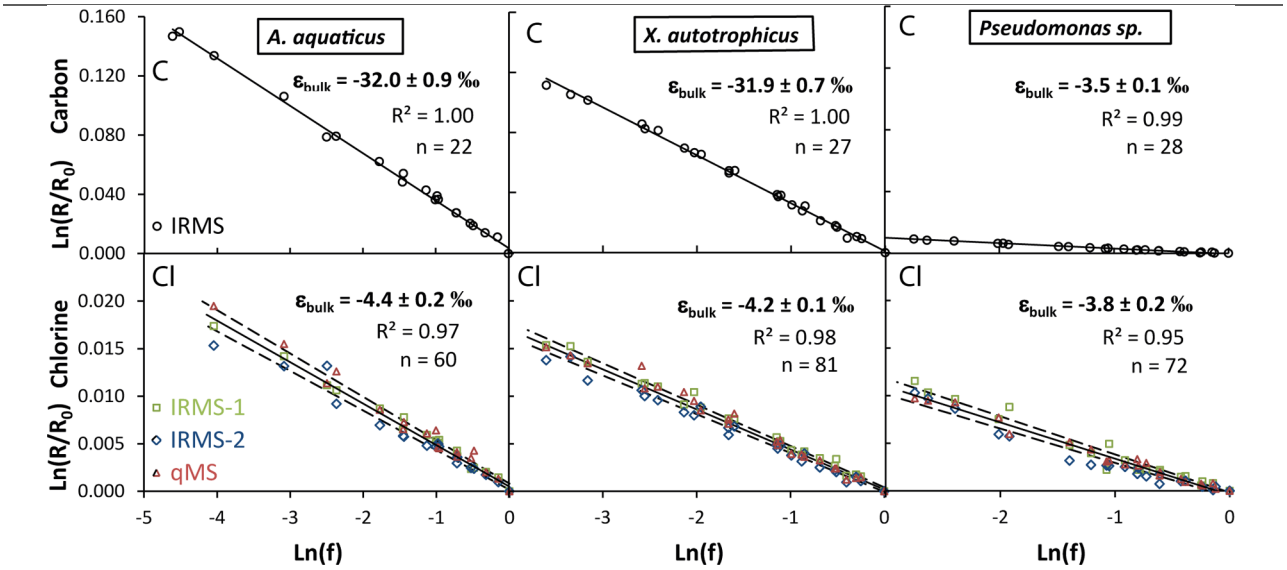


Figure 1. For each culture, C and Cl isotope data from triplicate experiments were combined in Rayleigh plots. For chlorine, isotope data from different instruments were also combined. The uncertainty for the carbon and chlorine ε values corresponds to the ±95% confidence interval calculated from the standard deviation of the regression slope. For chlorine, dashed lines represent the 95% confidence interval of regression parameters.

Therefore, observable ε_{bulk} values have to be converted into AKIEs to obtain information about the underlying reaction mechanisms.[21] For the calculation of AKIEs, a hypothesis about the reaction mechanism, or assumed reaction mechanism, is necessary. The effects of nonreacting positions within the molecule, as well as of intramolecular competition, are then taken into account using eqs 4 and 5,[21] respectively

$$\varepsilon_{rp} \approx \frac{n}{x} \times \varepsilon_{bulk} \quad (4)$$

$$AKIE_{C,Cl} = \frac{1}{z\varepsilon_{rp} + 1} \quad (5)$$

where ε_{rp} is the isotopic fractionation at the reactive position, n is the number of atoms of the element considered, x is the number of reactive sites, and z is the number of identical reactive sites undergoing intramolecular competition. In symmetric molecules such as 1,2-DCA, all atoms are in equivalent reactive positions ($n = x$), and therefore, ε_{rp} is directly obtained from eq 2.[21] The isotope fractionation values were quantified by least-squares linear regression according to eq 2 without forcing the regression to the origin.[39] As demonstrated by Elsner and Hunkeler,[27] the Rayleigh equation (eq 2) can also be applied to calculate the isotopic fractionation of chlorine despite the natural abundance of ^{37}Cl being higher than that of ^{13}C.

RESULTS AND DISCUSSION

Chlorine Isotope Fractionation Values from Different Instruments. Chlorine isotope fractionation values (ε^{Cl}_{bulk}) measured with different instruments are compared in Table 1. Results show excellent regressions (i.e., $r^2 \geq 0.95$) for the data from triplicate experiments when measured on the same instrument (entries in Table 1). In contrast, the variation was greater when data from different instruments were compared. The ε^{Cl}_{bulk} values from different instruments were compared using paired *t* tests conducted with SigmaPlot. The *p* values were above a significant level of 0.05, with the exception of the *p* value for *X. autotrophicus* between the IRMS-1 (ε^{Cl}_{bulk} = −4.3‰) and IRMS-2 (ε^{Cl}_{bulk} = −4.0‰) instruments (Table 1). This result indicates that, in general, there is no statistically significant difference in ε^{Cl}_{bulk} values at the 95% confidence level. The comparison of *p* values for different instrument pairs does not show systematic differences, suggesting that the variation in ε^{Cl}_{bulk} between laboratories could be in part related to minor effects during sample handling. The effect of scatter in data points at late stages of reaction on calculated ε^{Cl}_{bulk} (Figure 1) could also explain the small differences.[40] However, removing these data points did not significantly improve the agreement.

C and Cl Isotope Fractionation and Dual Isotope Slopes. Degradation experiments lasted between 12 h (*A. aquaticus*) and 21 h (*X. autotrophicus*), and 1,2-DCA transformation above 90% was reached for all the replicates. Carbon and chlorine ε_{bulk} values ($r^2 \geq 0.95$) (Figure 1) were

Table 2. Measured Carbon and Chlorine Isotopic Fractionation Values (in ‰) and Apparent Kinetic Isotope Effects

		observed				reported
	reaction mechanism	ε_{bulk}^{C} [a]	^{13}C-AKIE[b]	ε_{bulk}^{Cl} [a]	^{37}Cl-AKIE[b]	ε_{bulk}^{C}
A. aquaticus	S_N2	−32.0 ± 0.9	1.068 ± 0.002	−4.4 ± 0.2	1.0089 ± 0.0004	−31.9 to −32.4[16]
X. autotrophicus	S_N2	−31.9 ± 0.7	1.068 ± 0.002	−4.2 ± 0.1	1.0085 ± 0.0002	−28.7 to −33.0[16,35,41]
Pseudomonas sp.	C−H bond cleavage	−3.5 ± 0.1	1.0070 ± 0.0002	−3.8 ± 0.2	1.0038 ± 0.0002	−3.0 ± 0.2[16]

[a]See Rayleigh plots in Figure 1. The uncertainty corresponds to the 95% confidence interval calculated from the standard deviation of the regression slope. [b]Calculated according eq 5. The uncertainty was estimated by error propagation.

determined as indicated above (eq 2). Transformation of 1,2-DCA by *A. aquaticus* and *X. autotrophicus* by a haloalkane hydrolytic dehalogenase reaction resulted in the strong enrichment of ^{13}C in the remaining substrate, showing a δ^{13}C shift of approximately 98‰ at 95% degradation. The obtained ε_{bulk}^{C} and ε_{bulk}^{Cl} values for both cultures are the same within 95% confidence intervals, and ε_{bulk}^{C} values fall within the ranges determined from previous studies[16,35,41] (Table 2). Compared with that of carbon, a lower ε_{bulk}^{Cl} value of approximately −4.3‰ was determined for both cultures ($\varepsilon_{bulk}^{C}/\varepsilon_{bulk}^{Cl}$ = 7.4). Degradation of 1,2-DCA by *Pseudomonas* sp. strain DCA1 in an enzymatic monooxygenase reaction resulted in a smaller carbon ε_{bulk}^{C} (−3.5‰) compared to that of the nucleophilic (S_N2) reaction (Figure 1). This result is consistent with the previously reported value (Table 2).[16] For Cl, a ε_{bulk}^{Cl} value close to those associated with primary Cl isotopic effects was measured ($\varepsilon_{bulk}^{C}/\varepsilon_{bulk}^{Cl}$ = 0.9). This value is unusually high given that from a known mechanism a secondary isotope effect is expected (see further discussion in the next section).

A linear relation between δ^{13}C and δ^{37}Cl was obtained for all the strains ($r^2 \geq 0.97$), and strongly different slopes ($\Lambda = \Delta\delta^{13}C/\Delta\delta^{37}Cl \approx \varepsilon_{bulk}^{C}/\varepsilon_{bulk}^{Cl}$) were determined for the S_N2 reaction (Λ = 7.7 ± 0.2) and oxidation (Λ = 0.78 ± 0.03) (Figure 2). The slopes obtained from *X. autotrophicus* and *A. aquaticus* degradation experiments were the same within 95% confidence intervals. Therefore, the large difference in Λ among the investigated reactions allows the use of a dual isotope approach to identify the different pathways in the field. In contrast, a single element approach based only on carbon isotope data would lead to ambiguous interpretations because a certain extent of isotope fractionation (e.g., $\Delta\delta^{13}$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 (or an unknown combination of both). Unlike in lab experiments in which ε_{bulk}^{C} values can be determined,[16] insight into pathways would therefore be elusive in the field. The starkly contrasting trends of Figure 2 show how isotopic analysis of chlorine as a second element can resolve this issue. The large difference between determined slopes also allows the determination of whether both pathways occur in the field. In ideal situations, the proportion of both competing pathways could be estimated on the basis of dual isotope data.[42,43] A dual isotope analysis can also be helpful in microbial laboratory experiments for substantiating conclusions about prevailing mechanisms.

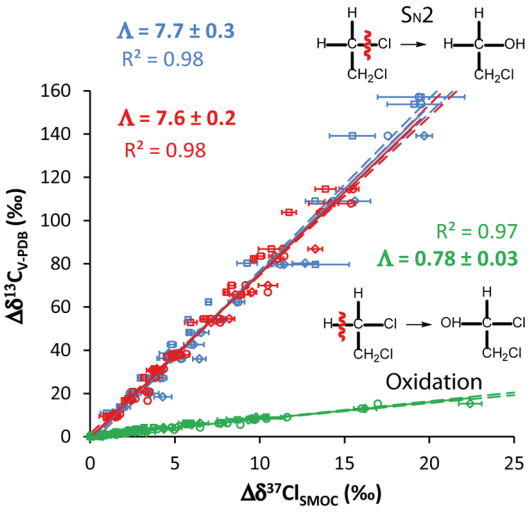


Figure 2. Carbon and chlorine δ isotope values from triplicate experiments and all used instruments (i.e., IRMS-1, IRMS-2, and qMS) were combined in a dual isotope plot. Symbols are as follows: blue for *A. aquaticus*, red for *X. autotrophicus*, green for *Pseudomonas* sp., circles for the IRMS-1 instrument, squares for the IRMS-2 instrument, and diamonds for the qMS instrument. The slopes of the linear regression lines (—) give the Λ values (±95% confidence intervals), and the dashed lines correspond to the 95% confidence intervals. Error bars of δ^{13}C values are smaller than the symbols.

Interpretation of ^{13}C- and ^{37}Cl-AKIEs for Oxidation and S_N2-Type Reactions. *Hydrolytic Dehalogenation (S_N2) Reaction.* Determined ε_{bulk} values were used to estimate the AKIEs (Table 2) according to eq 5, which assumes that secondary isotopic effects can be neglected. For the S_N2 reaction, ^{13}C-AKIEs were calculated using z = 2 because both C−Cl bonds compete for reaction. ^{13}C-AKIEs agreed well with the typical ^{13}C-KIE range for a S_N2 reaction (1.03−1.09)[21] (Scheme 1). Abe et al.[41] determined the ^{13}C-AKIE of 1,2-DCA in batch degradation experiments prepared with the cell free extract from *X. autotrophicus* GJ10. These authors obtained an average value (1.0597) close to the ^{13}C-AKIE observed in this study (1.068), suggesting that there was no significant masking of the intrinsic KIE during the transport of the compound through the cell membrane. This conclusion is in agreement with the Streitwieser limit for ^{13}C-KIE in C−Cl bonds (1.057)[44] and could explain in part the relatively narrow range of reported ε_{bulk}^{C} values (from −28.7 to −33.0‰) for both pure cultures using the haloalkane hydrolytic dehalogenase reaction (Table 2). Hirschorn et al.[9] measured a similar ^{13}C-AKIE (1.05) for 1,2-DCA in laboratory biodegradation experiments under nitrate reducing conditions by an enrichment culture from a contaminated site, which was interpreted as transformation via hydrolytic dehalogenation.

Like carbon isotope effects, ^{37}Cl-AKIEs were obtained from eq 5 with z = 2. The calculated ^{37}Cl-AKIE (1.009) corresponded well to the typical ^{37}Cl-KIE range for a S_N2 reaction but was very close to the upper end (1.006−1.009)[45] (Scheme 1). However, the ^{37}Cl-AKIE measured in this study is above the theoretical primary isotope effect for 1,2-DCA enzymatic dehalogenation reported by Lewandowicz et al. (^{37}Cl-KIE = 1.0065).[46] These authors also measured experimentally the leaving group ^{37}Cl-AKIE for 1,2-DCA (1.0045)

and 1-chlorobutane (1.0066) dechlorination catalyzed by haloalkane hydrolytic dehalogenase (extracted from *X. autotrophicus* GJ10). In this former study, the experimental ^{37}Cl-AKIE was determined by the isotopic analysis of the released Cl^- during 1,2-DCA dechlorination, and therefore, it represents the primary ^{37}Cl-AKIE. According to Lewandowicz et al.[46] and Paneth,[47] an explanation for the ^{37}Cl-AKIE of 1,2-DCA being lower than that of 1-chlorobutane could be that the dehalogenation step is reversible and the hydrolysis of the enzyme-bound intermediate is responsible for the overall irreversibility of the reaction. In addition, in a recent study that investigated biodegradation of 1,1-dichloroethane and 1,1,1-trichlorethane by whole cell and cell free extract systems,[48] ^{13}C-AKIEs for both chlorinated ethanes during degradation by cell free extracts were unexpectedly lower than those determined in whole cell experiments. The higher ^{37}Cl-AKIE of 1.009 compared to the theoretical primary ^{37}Cl-KIE could be explained by the contribution of a β-secondary isotopic effect given that, in our study, the ^{37}Cl/^{35}Cl ratios were measured in the remaining 1,2-DCA and secondary isotopic effects were neglected in the primary AKIE calculation (eq 5). The magnitude of the β-secondary ^{37}Cl-KIE can be estimated from the average of KIE_i in both Cl molecular positions according to eq 6.[12]

$$\varepsilon_{bulk}^{Cl} \approx \frac{1}{2}\left(\frac{1}{KIE_{primary}^{Cl}} + \frac{1}{KIE_{secondary}^{Cl}}\right) - 1 \quad (6)$$

Plugging in the ε_{bulk}^{Cl} value measured in our study (−4.3 ‰) and the reported theoretical primary Cl isotopic effect of 1.0065,[46,47] we estimated a β-secondary ^{37}Cl-KIE of 1.0021. Equation 6 assumes that ε_{bulk}^{Cl} is not significantly masked by nonfractionating rate-limiting processes preceding the reaction step. This is a likely assumption in our case judging by the relatively high carbon and chlorine AKIEs (see the discussion presented above and Table 2). Secondary ^{13}C-KIEs are generally much smaller than primary isotope effects, and therefore, they are usually omitted from ^{13}C-AKIE calculations.[21] However, for chlorine, secondary KIEs as large as primary isotope effects have been recently determined theoretically during S_N2 reactions that proceed with the transfer of chlorine between two heavy atoms.[45] In addition, in a recent experimental study of TCE multi-isotope fractionation during biotic reductive dechlorination,[32] daughter products depleted of ^{37}Cl relative to their immediate parent compounds were interpreted as evidence of significant secondary Cl effects related to the nucleophilic substitution reaction. The conclusions of these recent studies[32,45] support the hypothesis that a large β-secondary ^{37}Cl-KIE occurs during this study.

Oxidative C−H Bond Cleavage. As in the hydrolytic reaction, during oxidation both C atoms also compete for reaction, and thus, the ^{13}C-AKIE was calculated using $z = 2$ in eq 5 (Table 2). The obtained ^{13}C-AKIE agrees well with the typical ^{13}C-KIE for C−H bond cleavage (1.01−1.03)[21] (Scheme 1). Primary ^{13}C-KIEs generally increase with an increasing mass of the bonding partner (i.e., $^{13}C\text{-}KIE_{C-H} < {}^{13}C\text{-}KIE_{C-Cl}$).[21] For Cl, the unexpectedly high ε_{bulk}^{Cl} suggests the contribution of secondary ^{37}Cl-KIEs from both Cl atoms. In the absence of a primary chlorine isotopic effect, the secondary ^{37}Cl-AKIE can also be evaluated using eq 5. In this case, $z = 1$ because no specific bond containing Cl is broken, and there is, therefore, no intramolecular competition for this bond. The resultant secondary ^{37}Cl-AKIE (1.004) represents the average secondary isotope effect of all positions. This result supports the large β-secondary ^{37}Cl-KIE estimated above for the nucleophilic reaction. This finding suggests that significant secondary ^{37}Cl-KIEs are also associated with enzymatic oxidation via C−H bond cleavage. Until now, oxidative cleavage of a C−H bond has been believed to affect primarily the C−H bond and to leave chlorine substituents largely unchanged. This common assumption is challenged by the observed large chlorine isotope fractionation in C−H bond cleavage, where the involvement of a C−Cl bond would not be expected. This indicates an intriguing role of the chlorine atoms that remains to be resolved. The hypothetical contribution of chlorine isotope fractionation during binding of 1,2-DCA molecules to the enzyme could be an additional explanation.

Further insight might be obtained in future studies that address ^{2}H/^{1}H isotope analysis. Recently, Shouakar-Stash and Drimmie[49] developed an on-line methodology for H-CSIA of TCE and 1,2-*cis*-dichloroethene; however, this analytical method has not been implemented for chlorinated ethanes.

Implications for the Application of CSIA to Environmental Studies. One of the main applications of CSIA to field studies is the estimation of the extent and rate of contaminant biodegradation.[15,44,50] For this purpose, compound-specific ε_{bulk} values from laboratory experiments are necessary. For a given compound, different ε_{bulk} values are generally associated with distinct biodegradation pathways, which in turn are sometimes related to different subsurface redox environments. Therefore, redox conditions are usually used as criteria to constrain the range of reported ε_{bulk} values. However, for 1,2-DCA, different degradation pathways associated with distinct ε_{bulk} values may even be active under aerobic conditions. In addition, the hydrolytic dehalogenation pathway of 1,2-DCA has been observed under oxygen and nitrate reducing conditions alike (see above).[9] In this context, the identification of the active degradation pathway in the field is crucial for choosing the appropriate ε_{bulk} value for the quantification of degradation. This study demonstrates that dual isotope slopes are strongly different for nucleophilic substitution (S_N2) and oxidation (C−H bond cleavage) reactions, which opens the possibility of identifying them using a dual isotope approach. Following this approach, isotopic data from the field site can be directly and intuitively interpreted.

Significant secondary chlorine isotopic effects were determined for the investigated reactions. These results indicate that primary ^{37}Cl-AKIEs derived from CSIA could be higher than reported primary ^{37}Cl-KIEs (e.g., from computational studies) if secondary isotopic effects are omitted in the calculations. Hence, mechanistic interpretations based on the comparison with primary ^{37}Cl-KIEs should be made with caution.

Finally, chlorine ε_{bulk} values measured with three different instruments, two GC−IRMS instruments and one GC−qMS instrument, showed a fairly good agreement varying at most by ±3.8% (standard deviation of the mean). Even though the agreement was not perfect, these results lend confidence to the methods used and encourage the application of Cl-CSIA to investigate the fate of chlorinated compounds at contaminated sites. However, further research and methodological developments are still required to improve the agreement of Cl-CSIA data between different laboratories.

AUTHOR INFORMATION

Corresponding Author

*Centre d'Hydrogéologie et de Géothermie, Université de Neuchâtel, Rue Emile-Argand 11, CH-2000 Neuchâtel, Switzerland. E-mail: jordi.palau@ub.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to N. Jeanneret and Dr. E. Edwards for their help with the microcosm experiments. M.E. acknowledges funding from the German National Science Foundation (DFG), EL 266/3-1, as well as by the Initiative and Networking Fund of the Helmholtz Association. We thoroughly thank four anonymous reviewers for their comments on the manuscript.

REFERENCES

(1) Squillace, P. J.; Moran, M. J.; Lapham, W. W.; Price, C. V.; Clawges, R. M.; Zogorski, J. S. Volatile Organic Compounds in Untreated Ambient Groundwater of the United States, 1985−1995. *Environ. Sci. Technol.* **1999**, *33* (23), 4176−4187.

(2) ATSDR Toxicological Profile for 1,2-Dichloroethane, 2001. http://www.atsdr.cdc.gov/tfacts38.pdf (accessed June 7, 2013).

(3) Hage, J. C.; Hartmans, S. Monooxygenase-mediated 1,2-dichloroethane degradation by *Pseudomonas* sp. strain DCA1. *Appl. Environ. Microbiol.* **1999**, *65* (6), 2466−2470.

(4) 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.

(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), 976−983.

(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), 673−677.

(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) 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.

(9) 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), 1651−1657.

(10) 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.

(11) 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), 249−260.

(12) 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.

(13) Hofstetter, T. B.; Berg, M. Assessing transformation processes of organic contaminants by compound-specific stable isotope analysis. *TrAC, Trends Anal. Chem.* **2011**, *30* (4), 618−627.

(14) Hofstetter, T. B.; Schwarzenbach, R. P.; Bernasconi, S. M. Assessing Transformation Processes of Organic Compounds Using Stable Isotope Fractionation. *Environ. Sci. Technol.* **2008**, *42* (21), 7737−7743.

(15) 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.

(16) 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.

(17) Zwank, L.; Berg, M.; Elsner, M.; Schmidt, T. C.; Schwarzenbach, R. P.; Haderlein, S. B. New evaluation scheme for two-dimensional isotope analysis to decipher biodegradation processes: Application to groundwater contamination by MTBE. *Environ. Sci. Technol.* **2005**, *39* (4), 1018−1029.

(18) 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.

(19) 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 ($^{13}C/^{12}C$, $^{37}Cl/^{35}Cl$). *Environ. Sci. Technol.* **2013**, *47* (13), 6855−6863.

(20) Chartrand, M. M. G.; Hirschorn, S. K.; Lacrampe-Couloume, G.; Lollar, B. S. Compound specific hydrogen isotope analysis of 1,2-dichloroethane: Potential for delineating source and fate of chlorinated hydrocarbon contaminants in groundwater. *Rapid Commun. Mass Spectrom.* **2007**, *21* (12), 1841−1847.

(21) 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.

(22) Bigeleisen, J.; Wolfsberg, M. Theoretical and Experimental Aspects of Isotope Effects in Chemical Kinetics. *Adv. Chem. Phys.* **1958**, *1*, 15−76.

(23) 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.

(24) Sakaguchi-Soder, K.; Jager, J.; Grund, H.; Matthaus, F.; Schuth, S. Monitoring and evaluation of dechlorination processes using compound-specific chlorine isotope analysis. *Rapid Commun. Mass Spectrom.* **2007**, *21* (18), 3077−3084.

(25) Jin, B.; Laskov, C.; Rolle, M.; Haderlein, S. B. Chlorine isotope analysis of organic contaminants using GC-qMS: Method optimization and comparison of different evaluation schemes. *Environ. Sci. Technol.* **2011**, *45* (12), 5279−5286.

(26) 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.

(27) Elsner, M.; Hunkeler, D. Evaluating chlorine isotope effects from isotope ratios and mass spectra of polychlorinated molecules. *Anal. Chem.* **2008**, *80* (12), 4731−4740.

(28) Bernstein, A.; Shouakar-Stash, O.; Ebert, K.; Laskov, C.; Hunkeler, D.; Jeannottat, S.; Sakaguchi-Soder, K.; Laaks, J.; Jochmann,

(29) A.; Cretnik, S.; Jager, J.; Haderlein, S. B.; Schmidt, T. C.; Aravena, R.; Elsner, M. Compound-Specific Chlorine Isotope Analysis: A Comparison of Gas Chromatography/Isotope Ratio Mass Spectrom-

etry and Gas Chromatography/Quadrupole Mass Spectrometry Methods in an Interlaboratory Study. *Anal. Chem.* **2011**, *83* (20), 7624−7634.
(29) Wiegert, C.; Mandalakis, M.; Knowles, T.; Polymenakou, P. N.; Aeppli, C.; Macháčková, J.; Holmstrand, H.; Evershed, R. P.; Pancost, R. D.; Gustafsson, Ö. Carbon and Chlorine Isotope Fractionation During Microbial Degradation of Tetra- and Trichloroethene. *Environ. Sci. Technol.* **2013**, *47* (12), 6449−6456.
(30) Wiegert, C.; Aeppli, C.; Knowles, T.; Holmstrand, H.; Evershed, R.; Pancost, R. D.; Machackova, J.; Gustafsson, O. Dual Carbon-Chlorine Stable Isotope Investigation of Sources and Fate of Chlorinated Ethenes in Contaminated Groundwater. *Environ. Sci. Technol.* **2012**, *46*, 10918−10925.
(31) Hunkeler, D.; Abe, Y.; Broholm, M. M.; Jeannottat, S.; Westergaard, C.; Jacobsen, C. S.; Aravena, R.; Bjerg, P. L. Assessing chlorinated ethene degradation in a large scale contaminant plume by dual carbon-chlorine isotope analysis and quantitative PCR. *J. Contam. Hydrol.* **2011**, *119* (1−4), 69−79.
(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*, 9668−9677.
(33) Palau, J.; Marchesi, M.; Chambon, J. C.; Aravena, R.; Canals, A.; Binning, P. J.; Bjerg, P. L.; Otero, N.; Soler, A. Multi-isotope (carbon and chlorine) analysis for fingerprinting and site characterization at a fractured bedrock aquifer contaminated by chlorinated ethenes. *Sci. Total Environ.* **2014**, *475C*, 61−70.
(34) Lojkasek-Lima, P.; Aravena, R.; Parker, B. L.; Cherry, J. A. Fingerprinting TCE in a Bedrock Aquifer Using Compound-Specific Isotope Analysis. *Ground Water* **2012**, *50* (5), 754−764.
(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), 4870−4876.
(36) 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.
(37) Holt, B. D.; Sturchio, N. C.; Abrajano, T. A.; Heraty, L. J. Conversion of chlorinated volatile organic compounds to carbon dioxide and methyl chloride for isotopic analysis of carbon and chlorine. *Anal. Chem.* **1997**, *69* (14), 2727−2733.
(38) Staudinger, J.; Roberts, P. V. A critical compilation of Henry's law constant temperature dependence relations for organic compounds in dilute aqueous solutions. *Chemosphere* **2001**, *44* (4), 561−576.
(39) 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.
(40) Mundle, S. O. C.; Vandersteen, A. A.; Lacrampe-Couloume, G.; Kluger, R.; Lollar, B. S. Pressure-monitored headspace analysis combined with compound-specific isotope analysis to measure isotope fractionation in gas-producing reactions. *Rapid Commun. Mass Spectrom.* **2013**, *27* (15), 1778−1784.
(41) 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.
(42) 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), 4004−4010.
(43) 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.
(44) Aelion, C. M.; Hohëner, P.; Hunkeler, D.; Aravena, R. *Environmental isotopes in biodegradation and bioremediation*; CRC Press: Boca Raton, FL, 2010; p xiv.
(45) Swiderek, K.; Paneth, P. Extending Limits of Chlorine Kinetic Isotope Effects. *J. Org. Chem.* **2012**, *77* (11), 5120−5124.
(46) Lewandowicz, A.; Rudzinski, J.; Tronstad, L.; Widersten, M.; Ryberg, P.; Matsson, O.; Paneth, P. Chlorine kinetic isotope effects on the haloalkane dehalogenase reaction. *J. Am. Chem. Soc.* **2001**, *123* (19), 4550−4555.
(47) Paneth, P. Chlorine kinetic isotope effects on enzymatic dehalogenations. *Acc. Chem. Res.* **2003**, *36* (2), 120−126.
(48) 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), 7498−7503.
(49) 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.
(50) 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.