**Graphical abstract**

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**HIGHLIGHTS** *(3-5 bullets; 85 characters per bullet):*

* *Dehalogenimonas* transforms 1,1,2-trichloroethane (1,1,2-TCA) to vinyl chloride.
* Dual C-Cl isotope analysis applied for the first time for 1,1,2-TCA degradation.
* Significant C and Cl isotope fractionation during 1,1,2-TCA dichloroelimination.
* Calculated Λ can allow distinguishing 1,1,2-TCA degradation pathways in the field.

Dual carbon - chlorine isotope fractionation during dichloroelimination of 1,1,2-trichloroethane by an enrichment culture containing *Dehalogenimonas* sp.

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**ABSTRACT**

Chlorinated ethanes are frequent groundwater contaminants but compound specific isotope analysis (CSIA) has been scarcely applied to investigate their degradation pathways. In this study, dual carbon and chlorine isotope fractionation was used to investigate for the first time the anoxic biodegradation of 1,1,2-trichloroethane (1,1,2-TCA) using a *Dehalogenimonas*-containing culture. The isotopic fractionation values obtained for the biodegradation of 1,1,2-TCA were ɛC = -6.9 ± 0.4‰ and ɛCl = -2.7 ± 0.3‰. The detection of vinyl chloride (VC) as unique byproduct and a closed carbon isotopic mass balance corroborated that dichloroelimination was the degradation pathway used by this strain. Combining the values of δ13C and δ37Cl resulted in a dual element C-Cl isotope slope of Λ=2.5 ± 0.2‰. Investigation of the apparent kinetic isotope effects (AKIEs) expected for cleavage of a C-Cl bond showed an important masking of the intrinsic isotope fractionation. Theoretical calculation of Λ suggested that dichloroelimination of 1,1,2-TCA was taking place via simultaneous cleavage of two C-Cl bonds (concerted reaction mechanism). The isotope data obtained in this study can be useful to monitor natural attenuation of 1,1,2-TCA via dichloroelimination and provide insights into the source and fate of VC in contaminated groundwaters.

**Keywords:** *Dehalogenimonas*; dual isotope fractionation; dichloroelimination; organohalide-respiring bacteria; 1,1,2-trichloroethane.

1. **Introduction**

1,1,2-Trichloroethane (1,1,2-TCA) has been widely used as a solvent and chemical intermediate in the industry (**Pankow and Cherry, 1996**). Improper storage and accidental spills have contributed to 1,1,2-TCA being a frequent detected contaminant in groundwater at industrial facilities (**ATSDR, 1989)**. In the United States, it is ranked 166 out of 275 substances on the Priority List of Hazardous Substances based on a combination of its frequency, toxicity, and potential for human exposure (**ATSDR, 2015**).

Quantification of the distribution and fate of chlorinated contaminants and degradation products in the subsurface is a complex task since biological, chemical, and physical processes may affect them (**Němeček et al., 2017**). Biological transformation of 1,1,2-TCA is influenced by the intrinsic heterogeneity of natural environments that allows for different redox conditions to occur either spatially or temporally separated. Under anoxic conditions, reductive dechlorination is expected to be the prevailing mechanism to transform 1,1,2-TCA by two different biodegradation pathways: hydrogenolysis and dichloroelimination. In the case of dichloroelimination, two vicinal C-Cl bonds of 1,1,2-TCA are cleaved to produce vinyl chloride (VC), whereas during hydrogenolysis 1,1,2-TCA is sequentially transformed by single C-Cl bond cleavage to 1,2-dichloroethane (1,2-DCA) and monochloroethane (**Moe et al., 2016; Zhao et al., 2015**) (Fig. 1). The key organisms catalyzing hydrogenolysis and dichloroelimination are organohalide-respiring bacteria (OHRB), which can use 1,1,2-TCA as respiratory electron acceptor (**Leys et al., 2013**). To date, dichloroelimination of 1,1,2-TCA has been described for OHRB belonging to the genus *Dehalobacter* and *Dehalogenimonas* (**Grostern and Edwards, 2006; Mortan et al., 2017; Yan et al., 2009**), but hydrogenolysis only for *Desulfitobacterium* (**Zhao et al., 2015**). Under oxic conditions, no bacteria are currently known to use 1,1,2-TCA as growth substrate, but cometabolism of 1,1,2-TCA can occur during aerobic oxidation of methane, propane, butane, n-pentane, n-hexane or ammonia (**Frascari et al., 2006, 2008, 2013; Vannelli et al., 1990**). The only byproducts identified for aerobic cometabolism of 1,1,2-TCA include chloroacetic acid (which was sequentially oxidized to glyoxylic acid) and minor amounts of VC in microcosms containing a *Pseudomonas* sp. (**Castro and Belser, 1990**). Abiotic transformation of 1,1,2-TCA can produce a wide array of byproducts, including VC (**Patterson et al., 2016**), ethane (**Song and Carraway, 2005**) or 1,1-dichloroethene (1,1-DCE) (**Pagana et al., 1998**) (Fig.1).

Knowledge on degradation pathways occurring in an aquifer contaminated with 1,1,2-TCA is a key aspect to design suitable bioremediation strategies. However, this is a challenge when the site contains multiple chlorinated aliphatic hydrocarbons because the same daughter products of 1,1,2-TCA dechlorination can be formed from other precursors (i.e., VC is produced from anaerobic reductive dechlorination of DCE isomers or 1,2-DCA) (**Hunkeler et al., 2002**). It is important to note that VC, produced during biotic or abiotic reductive dichloroelimination of 1,1,2-TCA, is even much more toxic than 1,1,2-TCA.

Compound-specific isotope analysis (CSIA) has emerged in recent years as a technique with great potential to elucidate specific reaction pathways even if no products are detected (**Elsner, 2010**). The magnitude of carbon and chlorine kinetic isotope effects (KIEs) during contaminant degradation relies on the observation that lighter stable isotopes (i.e., 12C, 35Cl) react at faster rates than the heavier ones (i.e., 13C, 37Cl). For a given compound and reaction, single element isotope fractionationvalues (are determined in laboratory degradation experiments according to the Rayleigh equation. However,  values associated to biodegradation cannot be accurately measured in the field because other processes such as sorption or mixing through dispersion also affect contaminant concentration.

Two-dimensional CSIA brings the potential to overcome the limitation of single element isotope fractionation values to identify contaminant degradation pathways in the field. Combined changes in isotope ratios of two elements (i.e., 13C and 37Cl) for a given reactant generally correlate in a dual element isotope plot obtaining a slope ( = 13C / 37Cl) that reflects the isotope effects of both elements. Hence,  values may act as direct indicator for different initial reaction mechanisms. To interpret dual element CSIA data sets obtained from contaminated field sites, it is necessary to know experimental carbon and chlorine isotope enrichment factors and values derived from microbial strains catalyzing known transformation reactions (**Cretnik et al., 2013; Kuntze et al., 2016**). However, to our knowledge, chlorine isotope fractionation (Cl) and values are not available for 1,1,2-TCA. **Hunkeler et al. (2002)** showed that dichloroelimination of 1,1,2-TCA to VC in anaerobic microcosms inoculated with contaminated groundwater was accompanied of a relatively weak carbon isotopic fractionation of 1,1,2-TCA (εC = -2.0 ± 0.2‰). Recently, in a laboratory flow-through column experiment consisting of both biodegradable organic carbon and zero valent iron, εC changed from -14.6±0.7‰ to -0.72±0.12‰, being this last value assigned to anaerobic biodegradation (**Patterson et al., 2016**).

The main aims of this research were to measure for the first time dual C-Cl isotope fractionation and to determine the resultant value during biodegradation of 1,1,2-TCA with an anaerobic bacterial culture containing a *Dehalogenimonas* sp. This is valuable information i) to investigate the fate of 1,1,2-TCA in future biodegradation field studies and ii) to get insight into the underlying reaction mechanism involved in the dechlorination of 1,1,2-TCA. In addition, carbon isotope values of VC were measured to determine the product isotope pattern during biodegradation of 1,1,2-TCA.

1. **Materials and methods**
	1. **Biodegradation batch experiments**

A stable enrichment culture containing a *Dehalogenimonas* sp. described previously that transforms 1,1,2-TCA to VC via dichloroelimination (**Martín-González et al., 2015**) was used in batch experiments. Each microcosm consisted of 100 mL glass serum bottles containing 65 mL of a sterilized anoxic synthetic medium previously used to grow *Dehalococcoides mccartyi* strain CBDB1 (**Adrian et al., 2000**). This medium contained vitamins, trace elements, Na2S × 9 H2O and l-cysteine (0.2 mM each) as reducing agent, and as carbon source either sodium acetate (5 mM) or pyruvate (5 mM) as indicated. The serum bottles were sealed with Teflon-coated butyl rubber septa and aluminum crimp caps and gassed with N2/CO2 (4:1, v/v, 0.2 bar overpressure) and H2 (added to an overpressure of 0.4 bar). 1,1,2-TCA was added with a syringe from a stock solution in acetone to give an initial aqueous phase concentration of ~ 20 mol L-1; higher concentrations appeared to be inhibitory for this *Dehalogenimonas*-containing culture.

A total of 16 parallel incubations from the same inoculum were prepared at the same time. Half of these cultures contained acetate and the other half pyruvate as carbon source. Cultures were incubated at 25°C in the dark without shaking. Samples were collected for isotopic and concentration analyses at different extents of 1,1,2-TCA dechlorination. In order to control losses, abiotic transformations, and the transfer of compounds with the inoculum (previous growth in 1,2-dichloropropane, 1,2-DCP) or potential impurities from the stock solution, two types of controls were included in triplicate: (i) live controls without 1,1,2-TCA and (ii) abiotic controls containing the growth medium with 1,1,2-TCA but without inoculum.

* 1. **Analytical methods**
		1. ***Concentration and isotopic measurements.*** 1,1,2-TCA and VC concentrations in serum bottles were monitored along the experiment by taking 0.5 mL headspace (HS) samples with a 1.0 mL pressure-lock precision analytical syringe (Vici, U.S.) and injecting them in a gas chromatograph (GC) model 6890N (Agilent Technologies) equipped with a DB-624 column (30 m × 0.32 mm with 0.25 μm film thickness; Agilent Technologies) and a flame ionization detector (FID), as described elsewhere (**Palau et al., 2017**). Depending on the measured concentrations (expressed in μmol L–1 of liquid volume) the bottles were sacrificed at different extent of degradation stopping biological activity by adding 12 mL of an oxic, saturated H2SO4/Na2SO4 solution (pH=1).

Compound-specific carbon and chlorine isotope analyses were performed by HS-solid-phase micro-extraction (HS-SPME)-GC-isotope ratio mass spectrometry (GC-IRMS) as described elsewhere (**Palau et al., 2017**). δ13C analyses were performed in the *Centres Científics i Tecnològics de la Universitat de Barcelona* (CCiT-UB), Spain, while δ37Cl were carried out at *Isotope Tracer Technologies Inc.* (IT2), Canada. For analyzing chlorine isotope ratios of 1,1,2-TCA, the two most abundant fragment ions (*m/z* 97 and 99) were used, which correspond to isotopologue pairs (i.e., [**35Cl2**12C21H3]+ and [**37Cl35Cl**12C21H4]+, respectively) that differ by one heavy chlorine isotope. For 1,1,2-TCA, the intensities of the most abundant fragment ion peaks are much higher than those of the parent ion peaks. The raw δ37Cl values were calibrated to the standard mean ocean chloride (SMOC) scale using a two-point linear calibration. The standards were dissolved in water and measured similarly to the samples interspersed in the same sequence. Duplicate samples and standards were analyzed. The precision (1σ) on the analysis of standards was ≤0.5‰ for δ13C and ≤0.2‰ for δ37Cl.

* + 1. ***Isotope data evaluation.***Carbon and chlorine isotope ratios of 1,1,2-TCA were measured at natural abundance and were expressed using the -notation in per mil (eq. 1),

 (1)

where R is the isotope ratio of heavy (hE) to light (lE) isotopes of an element “E “(e.g., 13C/12C and 37Cl/35Cl). The relationship between isotope fractionation and the extent of 1,1,2-TCA biodegradation in laboratory experiments was evaluated by a modified form of the Rayleigh distillation equation (2)

 (2)

where hES0 is the initial isotopic composition of element “E” in a substrate “S” and hES is the isotopic composition at a remaining fraction “f” (f = CS/CS0). The compound-average isotope fractionation values () were quantified by least squares linear regression of eq. 2 without forcing the regression through the origin (**Scott et al., 2004**) and the uncertainty corresponds to the 95% confidence interval (C.I.) derived from the standard deviation of the regression slope. The Rayleigh equation can also be applied to calculate the isotopic fractionation of chlorine despite the higher natural abundance of 37Cl compared to 13C (**Elsner and Hunkeler, 2008**).

To evaluate the product carbon isotope fractionation pattern, the 13C of VC that was produced was calculated using eq. 3, where 13CP is the isotopic composition of the product “P” (i.e., VC) and is the estimated carbon isotopic fractionation of 1,1,2-TCA (eq. 2) (**Cretnik et al., 2014; Hunkeler et al., 2005**).

 (3)

For a given substrate, intrinsic KIEs during compound transformation are position specific whereas bulk values are calculated from compound-average isotope data (eq. 2). Therefore, observable bulk values must be converted into apparent KIEs (AKIEs) in order to obtain information about the underlying reaction mechanisms (**Elsner et al., 2005**). For the calculation and interpretation of AKIEs a hypothesis about the reaction mechanism, or assumed reaction mechanism, is necessary. The effects of non-reacting positions within the molecule, as well as of intramolecular competition, are then taken into account using equations 4 and 5, respectively (**Elsner et al., 2005**),

 (4)

 (5)

where rp is the isotopic fractionation at the reactive position, “n” is the number of atoms of the element considered, “x” is the number of these atoms at reactive sites (i.e., atoms that would experience isotope effects in the given reaction) and “z” the number of identical reactive sites undergoing intramolecular competition. These equations assume the absence of secondary isotope effects. For carbon, secondary isotope effects are usually insignificant (**Elsner et al., 2005**). For dichloroelimination of 1,1,2-TCA to VC, if the two C-Cl bonds are broken in sequence (i.e., *stepwise* dichloroelimination, single C-Cl bond cleavage at the first reaction step), assuming that the first bond cleavage is the rate determining step, then n = x = z = 2 and n = x = z = 3 for C and Cl, respectively, as all C and Cl atoms are in equivalent position and compete for reaction. On the other hand, if the two C-Cl bonds are broken simultaneously (i.e., *concerted* dichloroelimination), the average AKIEC and AKIECl for the two reacting positions were calculated since there is no intramolecular competition between them, n = x = 2, z = 1 and n = 3, x = 2, z = 1 for C and Cl, respectively. AKIEs that were calculated assuming *stepwise* or *concerted* dichloroelimination are referred hereafter as “” and “” and their uncertainty was calculated by error propagation.

For a given substrate and reaction, the dual C-Cl isotope slope () obtained from 13C vs 37Cl isotope plots can be expressed as follows (**Elsner, 2010** and references herein):

 (6)

**3. Results and discussion**

**3.1. Concentration and isotope patterns**

***3.1.1. Dechlorination of 1,1,2-TCA by a Dehalogenimonas-containing culture***. The anaerobic microcosms amended with pyruvate and acetate as carbon source lasted approximately 7 and 15 days, respectively, at which point the initial 1,1,2-TCA was transformed to VC via dichloroelimination. The concentration of 1,1,2-TCA in the abiotic controls (19.0 ± 0.5 mol L-1, ± 1, n = 5) remained at the initial concentration along the experiments, which indicates that compound losses through the caps during incubation were insignificant. The difference in the lag phase between acetate and pyruvate amended microcosms is not probably associated with the carbon source but to the inoculum source that was more enriched in the microcosms with pyruvate. No other volatile organic compounds were detected, especially 1,2-DCA was absent discarding 1,1,2-TCA hydrogenolysis. At different stages of 1,1,2-TCA degradation, isotope signatures of 1,1,2-TCA (δ13C and δ37Cl) and VC (δ13C) were measured for all the samples to determine the corresponding isotopic fractionation values of 1,1,2-TCA (εC and εCl) and the carbon isotope pattern of produced VC.

***3.1.2. Carbon isotope pattern of 1,1,2-TCA****.* The δ13C of 1,1,2-TCA in the abiotic controls remained constant through both experiments, with a total average value of -36.3 ± 0.6‰. In contrast, carbon isotopic composition of 1,1,2-TCA in the cultures became progressively enriched in 13C during its degradation reaching a δ13C value up to -14.3‰ when 96% of 1,1,2-TCA was degraded in both acetate- and pyruvate-containing media (Fig. 2). These results show that despite the differences in the lag phase and the inoculum source, no statistical difference in concentrations and carbon isotope values was observed for the experiments prepared with either acetate or pyruvate as carbon source. Isotopic data from both experiments were combined and the total carbon isotope composition of 1,1,2-TCA followed a Rayleigh trend (r2=0.9901, Fig. 3A) with an εC value of -6.9 ± 0.4‰ (95% C.I., n=16).

The similar isotope fractionation of 1,1,2-TCA for the microcosms amended with either acetate or pyruvate agrees with recent studies investigating isotopic fractionation of trichloroethene (TCE) under different growth conditions. **Harding et al. (2013)** showed that carbon isotope fractionation during TCE degradation by *Dehalococcoides*-containing cultures remained consistent despite a variety of temperature, nutrient, and cofactor-limiting conditions investigated. In addition, **Buchner et al. (2015)** studied the potential effects of metabolic adaptation on carbon and chlorine isotope fractionation of TCE during biodegradation by *Desulfitobacterium hafniesne* Y51. These authors reported similar bulk values for C and Cl isotopes under different growth conditions (i.e., cultures pre-grown with fumarate or TCE as electron acceptors) and enzyme quantity per cell and suggested that isotope fractionation was not affected.

***3.1.3. Carbon isotope pattern of VC.***In parallel to 1,1,2-TCA transformation, the δ13C of its degradation product (i.e., VC) was monitored. The δ13C of VC was initially depleted in 13C, in agreement with the normal isotope effect of 1,1,2-TCA, and shifted toward more positive values during the course of reaction reaching the initial value of 1,1,2-TCA once this was completely degraded (Fig. 2). As observed for 1,1,2-TCA, the carbon isotope data of VC from the experiments with acetate and pyruvate showed similar values (Fig. 2). This figure also shows that δ13C values of VC fitted very well with the expected product isotope trend determined according to eq. 3. The closed isotopic mass balance confirmed the absence of other relevant degradation products. Moreover, δ13C of VC never overpass the initial δ13C of 1,1,2-TCA suggesting that VC is not further degraded to non-chlorinated compounds such as ethene or ethane, which is consistent to its accumulation.

A different product isotope pattern was observed for degradation of 1,1,2-TCA in a previous study with microcosms constructed with aquifer material and groundwater (**Hunkeler et al., 2002**). These authors observed δ13C values of VC very enriched in 13C compared to those of 1,1,2-TCA towards the end of reaction, which was indicative of further degradation of VC to ethene via reductive dechlorination. Therefore, the results of the present study and **Hunkeler et al., 2002** illustrate the potential of the product carbon isotope pattern to investigate the fate of VC in sites impacted with 1,1,2-TCA. Analysis of ethene concentration can be used to evaluate the fate of VC in groundwater, provided that other potential precursors of ethene such as 1,2-DCA are not present at the site. However, assessing the fate of VC based solely on ethene concentration can be difficult because ethene can be transformed under both oxic and anoxic conditions to carbon dioxide and ethane, respectively (**Mundle et al., 2012**), highlighting the benefit of VC isotope analysis as complementary data.

***3.1.4. Chlorine isotope pattern of 1,1,2-TCA and dual C-Cl isotope approach*.** Chlorine isotope data of 1,1,2-TCA (δ37Cl) were obtained from the pyruvate amended microcosms. The δ37Cl of 1,1,2-TCA in the abiotic controls (-0.88 ± 0.2‰) did not change significantly during the experiment, while an enrichment in the heavy isotope (37Cl) during 1,1,2-TCA degradation following a Rayleigh trend (εCl = -2.7 ± 0.3‰, n=8, 95% C.I., Fig. 3B) was observed in the cultures. Chlorine isotope fractionation was much lower than for carbon, in agreement with the large primary carbon isotope effects expected for C-Cl bond cleavage (**Elsner et al., 2005**). The measurement of chlorine isotope ratios enabled for the first time a dual C-Cl isotope approach for biodegradation of 1,1,2-TCA. A very good linear correlation (r2 = 0.994) was obtained when δ13C and δ37Cl were combined in a dual element isotope plot showing a slope (Λ) of 2.5 ± 0.2 (95% C.I., Fig. 4).

A recent study on 1,2-DCA showed different Λ values during dichloroelimination by *Dehalogenimonas*- and *Dehalococcoides*-containing cultures, suggesting that a dual C-Cl isotope approach could help to identify the microbial taxa responsible for anaerobic biodegradation of 1,2-DCA in the field (**Palau et al., 2017**). This information is particularly important for 1,1,2-TCA given that, in contrast to *Desulfitobacterium* (**Zhao et al., 2015**) (Fig. 1), its degradation by *Dehalogenimonas* can result in an accumulation of the highly toxic VC in groundwater. Therefore, comparison of the Λ value obtained for *Dehalogenimonas* in the present study with those obtained for 1,1,2-TCA degradation by other bacteria in future studies might help to investigate the fate of 1,1,2-TCA and to predict potential accumulation of VC in contaminated sites.

**3.2. Isotope effects and insight into dichloroelimination mechanisms of 1,1,2-TCA**

Significant variation on reported bulk carbon isotope fractionation during biodegradation of 1,1,2-TCA is observed (Table 1). The εC value of -6.9 ± 0.4‰ determined in this study is significantly larger than that previously reported, -2.0 ± 0.2‰ from microcosms constructed with anaerobic aquifer material and groundwater (**Hunkeler et al., 2002**). In addition, a much lower εC value of -0.7 ± 0.1‰ was determined by Patterson et al. 2016, which was attributed to biodegradation in a laboratory column consisted of both zero valent iron Fe(0) and biodegradable organic carbon. Interestingly, microbiological data from this laboratory column suggested that a co-culture composed by *Desulfitobacterium* and *Dehalococcoides* was responsible for the sequential degradation of 1,1,2-TCA to ethene. The enzymatic mechanism of *Desulfitobacterium* sp. strain PR to transform 1,1,2-TCA to 1,2-DCA via hydrogenolysis differs from the production of VC via dichloroelimination in our *Dehalogenimonas*-containing culture which could explain the difference on carbon isotope fractionation observed in both studies (Fig. 1). A simultaneous cleavage of two C-Cl bonds via *concerted* dichloroelimination of 1,1,2-TCA might result theoretically in a larger bulk εC value compared to hydrogenolysis, where a single C-Cl bond is broken at the initial reaction step. However, the occurrence of isotope-masking leading to smaller εC values cannot be excluded. In this case, if preceding (rate-limiting) steps exhibit small or no isotope fractionation, the observable isotope effect will be smaller (i.e., masked) than the intrinsic isotope effect.

To address in more detail whether dichloroelimination of 1,1,2-TCA by *Dehalogenimonas* proceeds via a *stepwise* or *concerted* mode, AKIE values were calculated according to eq. 4 and 5 as it was previously done with the same *Dehalogenimonas* containing enrichment for 1,2-DCP (**Martín-González et al., 2015**) or 1,2-DCA (**Palau et al., 2017**, see also Table 1). Assuming stepwise or concerted mode, carbon AKIEs obtained for 1,1,2-TCA (AKIECstepwise = 1.0138 ± 0.0008 and AKIECconcerted = 1.0069 ± 0.0004, respectively) were much below the Streitweiser limit of KIEC for complete C-Cl bond cleavage (1.057) and the realistic value of 50% bond cleavage (1.029) (**Elsner et al., 2005**), making both modes feasible, but showing important masking of intrinsic isotope fractionation. For chlorine, AKIEs determined for both mechanisms (AKIEClstepwise = 1.0082 ± 0.0009 and AKIEClconcerted = 1.0041 ± 0.0005), were also below the Streitweiser limit for C-Cl bond cleavage (1.013).

Apart from theoretical Streitweiser limits, isotopic fractionation values and derived AKIEs from abiotic reactions are often considered closest to the intrinsic isotope effects. Abiotic reductive dechlorination of 1,1,2-TCA was suggested in the same above-mentioned Fe(0) column study but without the organic carbon amendment (Patterson et al. 2016). In that case, an AKIE for stepwise mode of 1.0246 can be calculated from the reported εC value (−12 ± 5‰). This AKIE value is within the range (AKIECstepwise = 1.0158 to 1.0326) previously available for abiotic reductive dechlorination of 1,1,1-TCA and other polychlorinated ethanes, 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA), pentachloroethane (PCA) and hexachloroethane (HCA) by Cr(II), Fe(0) and Cu and Fe mixtures (**Elsner et al., 2007; Hofstetter et al., 2007; Palau et al., 2014**). Chlorine isotope effects (AKIEClstepwise = 1.0125 to 1.0207) were also reported by **Hofstetter et al., 2007** and **Palau et al., 2014**. The reported carbon and chlorine AKIEs for abiotic reductive dechlorination of chlorinated ethanes (via single C-Cl bond cleavage at the first reaction step) are higher than those determined for 1,1,2-TCA dichloroelimination in this study assuming either stepwise or concerted scenarios. Therefore, mechanistic interpretations are challenged by the relatively low observable bulk isotope effects of 1,1,2-TCA. The occurrence of isotope-masking effects can sometimes complicate the identification of the underlaying reaction mechanism since derived AKIEs may then be no longer characteristic of a certain reaction (**Elsner et al., 2005**). However, an improved interpretation might be possible by comparing dual C-Cl isotope slopes (see below).

A large isotope fractionation masking such that of 1,1,2-TCA during degradation by *Dehalogenimonas* in this study was also observed for *Dehalobacter*-containing mixed culture degrading 1,1,1-TCA versus 1,1-DCA (**Sherwood Lollar et al., 2010**). In particular, the large intrinsic kinetic isotope effect expected for cleavage of a C-Cl bond was almost completely masked during 1,1,1-TCA biodegradation by both whole cells and cell-free extracts, while for 1,1-DCA the reduction was only roughly 50%. These effects were not attributable to transport effects across the cell membrane, rather than to significant differences in the kinetics of the enzymes catalyzing chlorinated ethane degradation.

**3.3. Reaction mechanism insight from dual C-Cl plot.**

An important advantage of values compared to bulk values (and derived AKIEs) is that the magnitude of the latter can be significantly affected by isotope-masking processes. Since isotope-masking affect both elements to a similar extent, the dual element isotope slopes remain largely unaltered (**Elsner, 2010**). For 1,1,2-TCA, the lack of degradation studies including both carbon and chlorine isotope data makes not possible a comparison of the value determined for *Dehalogenimonas* in this study with values for different reactions (biotic and abiotic) and microbial strains. However, values for a new compound like 1,1,2-TCA can be predicted based on the expected KIEs for carbon and chlorine according to eq 6, and it can be then compared to the experimentally determined value for *Dehalogenimonas*.

Assuming *concerted* dichloroelimination of 1,1,2-TCA (n = x = 2, z = 1 and n = 3, x = 2, z = 1 for C and Cl, respectively, see above), the carbon and chlorine isotope effects determined in a recent study (**Palau et al., 2017**) for reductive dichloroelimination of 1,2-DCA by *Dehalogenimonas* were used in eq. 6 (AKIECconcerted = 1.024 ± 0.003 and AKIEClconcerted = 1.0121 ± 0.0008, see Table 1). These authors postulated a concerted character of the reaction based on determined carbon isotope effects. As a result, a value of 2.98 was obtained, which is similar to the experimental value of 2.5 ± 0.2 (Fig. 4). In contrast, if a *stepwise* dichloroelimination of 1,1,2-TCA is assumed (n = x = z = 2 and n = x = z = 3 for C and Cl, respectively), a very different value of 1.01 is obtained. In this case, the average carbon and chlorine isotope effects for 1,1,2,2-TeCA, PCA and HCA during abiotic dichloroelimination by Cr(II) via sequential **-elimination of two chlorine atoms were considered (AKIECstepwise = 1.026 ± 0.005 and AKIEClstepwise = 1.017 ± 0.004, see Table 1) (**Hofstetter et al., 2007**). In addition, a smaller value of 0.66 was obtained in case the AKIEs estimated for reduction of 1,1,1-TCA by Fe(0) via single electron transfer are used in the calculations (AKIECstepwise = 1.0158 ± 0.0008 and AKIEClstepwise = 1.0160 ± 0.0006, see Table 1). Therefore, the comparison of the experimental value of 1,1,2-TCA with those expected for stepwise and concerted mechanisms according to eq. 6 suggests that a concerted dichloroelimination is more likely, highlighting the benefit of using a dual C−Cl isotope approach. This result is in agreement with previous studies of 1,2-DCP and 1,2-DCA biodegradation by *Dehalogenimonas* suggesting a concerted dichloroelimination pathway (**Martín-González et al., 2015; Palau et al., 2017**). Identification of the underlying transformation mechanism controlling isotope fractionation can be valuable information to improve the characterization of reductive dehalogenases. In addition, an eventual identification of different dichloroelimination mechanisms of 1,1,2-TCA (i.e., concerted vs stepwise) by distinct microbial strains might indicate the existence of diverse reductive dehalogenases with similar function but likely different structure. For 1,2-DCA, the isotopic differences observed by **Palau et al. (2017)** between *Dehalogenimonas* and *Dehalococcoides* containing cultures on the *concerted* dichloroelimination mechanism were associated to a distinct interaction mode between cobalamin dependent enzymes rather than two different reaction pathways (i.e., stepwise vs concerted). The same isotopic results and conclusions were validated by **Franke et al. (2017)** with two pure *Dehalococcoides mccartyi* strains (195 and BTF08).

**4. Conclusions**

1,1,2-TCA is a frequent groundwater contaminant but surprisingly only few studies applying CSIA have been reported so far. Our work provides the first application of dual isotope fractionation to investigate the anaerobic biodegradation of 1,1,2-TCA. The stable isotope data obtained in this study during the dichloroelimination of 1,1,2-TCA can be potentially helpful in monitoring the fate of this pollutant in contaminated environments. In addition, the carbon isotope pattern of VC obtained in our enrichment enlightens its potential use to identify the dominant VC production mechanism and predict further transformation of this toxic compound. The single element kinetic isotope effects could not provide conclusive information about the reaction mechanism involved in 1,1,2-TCA dichloroelimination (concerted or stepwise); however, the dual-element approach can reduce interpretation bias due to isotope-masking effects overcoming this limitation and pointing to more likely concerted mechanism. Further investigations on carbon and chlorine isotope fractionation with bacteria catalyzing alternate degradation pathways (i.e., hydrogenolysis) will allow the comparison between microbial dechlorination reactions of 1,1,2-TCA.

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**Figure 1**. Degradation pathways of 1,1,2-TCA: (a) dehydrochlorination, (b) hydrogenolysis, (c) dichloroelimination, (d) hydrolysis. Numbers indicate the dechlorinating agent: (1) base mediated abiotic reaction in aqueous solution (Pagana et al. 1998); (2) *Desulfitobacterium* sp. strain PR (Zhao et al. 2015); (3) *Dehalobacter* and *Dehalogenimonas* spp (Grostern and Edwards, 2006; Mortan et al. 2017, Yan et al. 2009); (4) nanosized zero-valent iron (Song and Carraway, 2005); (5) zero valent iron and zinc (Patterson et al. 2016); (6) *Pseudomonas* sp. (Castro and Belser, 1990). Bold arrow: biotic reaction; dashed arrow: abiotic reaction.

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**Figure 2.** Concentration and carbon isotope patterns of 1,1,2-TCA (circles) and VC (triangles) during dichloroelimination of 1,1,2-TCA in a *Dehalogenimonas*-enrichment culture prepared with either acetate (empty symbols) or pyruvate (filled symbols) as carbon source. The error bars show the one standard deviation (1σ) for duplicate measurements. For isotope values the error bars are smaller than the symbols. The average δ13C of 1,1,2-TCA in the controls (dashed line) and models fit to isotope data from the substrate (eq 2, black solid line) and product (eq 3, grey solid line) are shown.

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**Figure 3.** Double logarithmic plot according to the Rayleigh equation (eq 2) of the carbon (A) and chlorine (B) isotope ratios versus the residual concentration of 1,1,2-TCA during dichloroelimination by a *Dehalogenimonas*-containing culture prepared with either acetate (empty symbols) or pyruvate (filled symbols) as carbon source. The error bars show the one standard deviation (1σ) for duplicate measurements and doted lines represent the 95% C.I. of the linear regression determined by SigmaPlot.

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**Figure 4.** Dual C-Cl isotope plot during dichloroelimination of 1,1,2-TCA in a *Dehalogenimonas*-containing enrichment culture. The error bars show the one standard deviation (1σ) for duplicate measurements. For C isotope values the error bars are smaller than the symbols. Doted lines represent the 95% C.I. of the linear regression determined by SigmaPlot.

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**Table 1.** Comparison of ε and AKIE values for C and Cl isotopes assuming either stepwise or concerted reductive dechlorination of chlorinated ethanes and propanes.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **AKIEC** |  | **AKIECl** |  |  |
| **Compound** | **Degradation experiment** | **ɛC (‰)** | **Stepwise** | **Concerted** | **ɛCl (‰)** | **Stepwise** | **Concerted** | **Λ** | **Reference** |
| 1,1,2-TCA | *Dehalogenimonas* -containing culture | -6.9 ± 0.4 | 1.0138 ± 0.0008 | 1.0069 ± 0.0004 | -2.7 ± 0.3 | 1.0082 ± 0.0009 | 1.0041 ± 0.0005 | 2.5 ± 0.2 | This study |
| 1,1,2-TCA | Anoxic microcosms | -2.0 ± 0.2 | 1.0040\* | 1.0020\* | n.m. |  |  | n.m. | Hunkeler et al. (2002) |
| 1,1,2-TCA | Laboratory column 20% (w/w) Fe(0)/organic carbon amendment | −14.6 ± 0.7 to−0.7 ± 0.1 | 1.0301to1.0014\* | 1.0148to1.0007\* | n.m |  |  | n.m | Patterson et al. (2016) |
| 1,1,2-TCA | Abiotic laboratory column with Fe(0) without organic carbon amendment | −12 ± 5 | 1.0246\* | 1.0121\* | n.m |  |  | n.m | Patterson et al. (2016) |
| 1,1,1-TCA | abiotic by Cr(II), Fe(0) and Cu and Fe mix | -13.6 ± 0.5to-15.8 ± 0.6 | 1.028 ± 0.001to1.033 ± 0.001 | n.a | n.m. |  |  | n.m. | Elsner et al. (2007) |
| 1,1,1-TCA | abiotic by Fe(0) | -7.8 ± 0.4 | 1.0158 ± 0.0008 | n.a | -5.2 ±0.2 | 1.0160 ± 0.0006 |  | 1.5 ± 0.1 | Palau et al. (2014) |
| 1,1,1-TCA | abiotic degradation mediated by biotic FeS formation in bioaugmented microcosms | −10.3 to −14.0 |  |  | n.m. |  |  | n.m. | Broholm et al. (2014) |
| 1,1,1-TCA | *Dehalobacter*-containing culture (whole cell and cell-free extracts) | -1.8 ± 0.3-0.8 ± 0.3 | 1.0036 ± 0.00061.0016 ± 0.0006 | n.a | n.m. |  |  | n.m. | Sherwood Lollar et al. (2010) |
| 1,2-DCA | *Dehalococcoides mccartyi* strains (195 and BTF08) | -28.4 ± 3.7-30.9 ± 3.6 | 1.059 ± 0.0081.066 ± 0.008 | 1.029 1.031 | -4.6 ± 0.7-4.2 ± 0.5 | 1.009 ± 0.0011.009 ± 0.001 | 1.0051.004 | 6.9 ± 1.27.1 ± 0.2 | Franke et al. (2017) |
| 1,2-DCA | *Dehalococcoides mccartyi* strains (195 and BTF08) | -29.0 ± 3.0-30.8 ± 1.3 | 1.0621.066 | 1.0301.033 | n.m |  |  | n.m | Schmidt et al. (2014) |
| 1,2-DCA | *Dehalococcoides*-containing culture | −33.0 ± 0.4 | 1.0707 ± 0.0009 | 1.0341 ± 0.0004 | −5.1 ± 0.1 |  | 1.0051 ± 0.0001\* | 6.8 ± 0.2 | Palau et al. (2017) |
| 1,2-DCA | *Dehalogenimonas*-containing culture | −23 ± 2 | 1.048 ± 0.004 | 1.024 ± 0.003 | −12.0 ± 0.8 |  | 1.0121 ± 0.0008\* | 1.89 ± 0.02 | Palau et al. (2017) |
| 1,2-DCA | Anoxic microcosms | -32 ± 1 | 1.069 ± 0.002\* | 1.033 ± 0.001\* | n.m |  |  | n.m | Hunkeler et al. (2002) |
| 1,2-DCA | abiotic by Zn(0) | -29.7 ± 1.5 | 1.06 – 1.07 | 1.03 | n.m. |  |  | n.m. | Vanstone et al. (2008) |
| 1,1-DCA | *Dehalobacter*-containing culture (whole cell and cell-free extracts) | -10.5 ± 0.6 and-7.9 ± 0.9 | 1.021 ± 0.002and1.016 ± 0.002 |  | n.m. |  |  | n.m. | Sherwood Lollar et al. (2010) |
| 1,1,2,2-TeCA | abiotic by Cr(II), Fe(0) and Cu and Fe mix | -17.0 ± 0.6 to -19.3 ± 0.7 | 1.035 ± 0.001 to1.040 ± 0.001 | 1.0173 ± 0.0006 to1.0196 ± 0.0008 | n.m. |  |  | n.m. | Elsner et al. (2007) |
| 1,1,2,2-TeCA | Abiotic by Cr(II) | -12.7 ± 1.2 | 1.026 ± 0.001 | 1.013\* | n.m |  |  | n.m | Hofstetter et al. (2007) |
| PCA | Abiotic by Cr(II) | -14.7 ± 0.6  | 1.0303 ± 0.0006 | 1.0149\* | n.m |  |  | n.m | Hofstetter et al. (2007) |
| HCA | Abiotic by Cr(II) | -10.4 ± 0.5 | 1.0212 ± 0.0005 | 1.0105\* | n.m |  |  | n.m | Hofstetter et al. (2007) |
| 1,2-DCP | Culture RC containing *Dehalococcoides* | -10.8 ± 0.9 | 1.033 ± 0.003 | 1.016 ± 0.001 | n.m |  |  | n.m | Fletcher et al. (2009) |
| 1,2-DCP | Culture KS containing *Dehalococcoides* | -11.3 ± 0.8 | 1.033 ± 0.003 | 1.017 ± 0.001 | n.m |  |  | n.m | Fletcher et al. (2009) |
| 1,2-DCP | Culture BRcontaining *Dehalogenimonas* | -15.0 ± 0.7 | 1.045 ± 0.002 | 1.023 ± 0.001 | n.m |  |  | n.m | Martín-González et al. (2015) |

n.m. not measured, n.a. not applicable. \* Approximated values calculated from epsilon according to Elsner et al., 2005.

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