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Dual C–Cl isotope fractionation offers potential to assess biodegradation of 1,2-dichloropropane and 1,2,3-trichloropropane by *Dehalogenimonas* cultures

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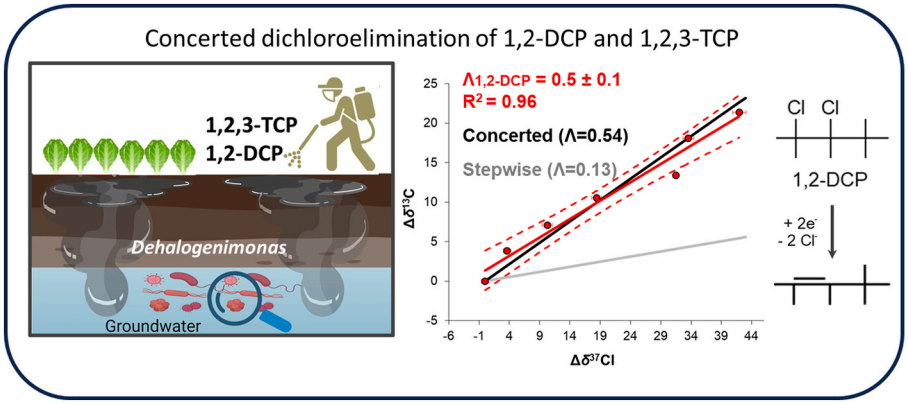
HIGHLIGHTS

- *Dehalogenimonas* coupled growth with dechlorination of 1,2-DCP and 1,2,3-TCP.
- Same set of reductive dehalogenases were involved in their biodegradation.
- Dechlorination of 1,2-DCP and 1,2,3-TCP was assessed using dual isotope analysis.
- Concerted dichloroelimination mechanism is more likely for both compounds.
- Calculated Λ allows distinguishing their degradation pathways in the field.

GRAPHICAL ABSTRACT



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ABSTRACT

1,2-dichloropropane (1,2-DCP) and 1,2,3-trichloropropane (1,2,3-TCP) are hazardous chemicals frequently detected in groundwater near agricultural zones due to their historical use in chlorinated fumigant formulations. In this study, we show that the organohalide-respiring bacterium *Dehalogenimonas alkenigignens* strain BRE15 M can grow during the dihaloelimination of 1,2-DCP and 1,2,3-TCP to propene and allyl chloride, respectively. Our work also provides the first application of dual isotope approach to investigate the anaerobic reductive dechlorination of 1,2-DCP and 1,2,3-TCP. Stable carbon and chlorine isotope fractionation values for 1,2-DCP ($\varepsilon_C = -13.6 \pm 1.4$ ‰ and $\varepsilon_{Cl} = -27.4 \pm 5.2$ ‰) and 1,2,3-TCP ($\varepsilon_C = -3.8 \pm 0.6$ ‰ and $\varepsilon_{Cl} = -0.8 \pm 0.5$ ‰) were obtained resulting in distinct dual isotope slopes ($\Lambda_{12DCP} = 0.5 \pm 0.1$, $\Lambda_{123TCP} = 4 \pm 2$). However direct comparison of $\Lambda_{C\text{-}Cl}$ among different substrates is not possible and investigation of the C and Cl apparent kinetic

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isotope effects lead to the hypothesis that *concerted* dichloroelimination mechanism is more likely for both compounds. In fact, whole cell activity assays using cells suspensions of the *Dehalogenimonas*-containing culture grown with 1,2-DCP and methyl viologen as electron donor suggest that the same set of reductive dehalogenases was involved in the transformation of 1,2-DCP and 1,2,3-TCP. This study opens the door to the application of isotope techniques for evaluating biodegradation of 1,2-DCP and 1,2,3-TCP, which often co-occur in groundwaters near agricultural fields.

1. Introduction

The chlorinated propanes, 1,2,3-trichloropropane (1,2,3-TCP) and 1,2-dichloropropane (1,2-DCP), are toxic substances of anthropogenic origin which are classified as probable human carcinogens by the U.S. Environmental Protection Agency (EPA, 2014). Both compounds are often detected together in groundwater near agricultural areas, owing to their historical use as components in chlorinated fumigant formulations for agricultural purposes (i.e. products Telone and DD-mix). For instance, 1,2,3-TCP was detected in 6.5% of 1237 wells sampled in California by the U. S. Geological Survey, and in 49% of 1,2,3-TCP detections, 1,2-DCP co-occurred (Burow et al., 2019). In addition to contamination by 1,2-DCP and 1,2,3-TCP from diffuse sources linked to their use in agricultural practices, contamination of soil and groundwater for both compounds also occurred as point source pollution due to their improper disposal or accidental spills (Samin and Janssen, 2012).

Remediation of 1,2,3-TCP and 1,2-DCP contaminated sites with organohalide-respiring bacteria (OHRB) is a feasible strategy in anoxic environments. This potential relies on the peculiar respiratory machinery of these anaerobic microorganisms that use halogenated compounds as electron acceptors and mainly hydrogen as electron donor to yield energy for growth. To date, bacteria belonging to the genera *Dehalobacter*, *Desulfitobacterium, Dehalogenimonas* and *Dehalococcoides* are known to respire 1,2-DCP to the innocuous propene (de Wildeman et al., 2003; Schlötelburg et al., 2002; Ritalahti and Löffler, 2004), but only *Dehalogenimonas* sp. are reported to transform 1,2,3-TCP. From all *Dehalogenimonas* strains characterized so far, *D. lykanthroporepellens* strains BL-DC-8 and BL-DC-9 (Moe et al., 2009; Samin and Janssen, 2012; Yan et al., 2009), *D. formicexedens* NSZ-14T (Key et al., 2017), and *D. alkenigignens* strains IP3-3 (Key et al., 2016) and BRE15 M (Trueba-Santiso et al., 2021) are capable of transforming both 1,2-DCP and 1,2,3-TCP. *Dehalogenimonas* sp. strain WBC-2 was reported to dechlorinate 1,2-DCP, but not 1,2,3-TCP (Molenda et al., 2016) and '*Ca.* D. etheniformans' strain GP was tested for both substrates with a negative result (Yang et al., 2017). In all the 1,2,3-TCP-transforming strains, allyl chloride (3-chloro-1-propene) was the main dechlorination byproduct. At the core of these reductive dechlorination processes in OHRB are the reductive dehalogenase (RdhA) proteins, which are membrane-bound enzymes linked to other cellular components that catalyze dehalogenation reactions to generate cellular energy. A RdhA involved in the 1,2-DCP-to-propene dechlorination in *Dehalococcoides mccartyi* and *Dehalogenimonas*, which was denominated DcpA, was identified using blue native polyacrylamide gel electrophoresis coupled with in-gel enzymatic assays and peptide sequencing (Padilla-Crespo et al., 2014). The biochemical confirmation of the RdhA involved in the dechlorination of 1,2,3-TCP is still missing but transcriptional analyses showed that multiple *rdhA* genes were transcribed simultaneously when *D. lykanthroporepellens* strain BL-DC-9 was growing on 1,2,3-TCP (Mukherjee et al., 2014).

Compound-specific isotope analysis (CSIA) has proven to be a robust technique that permits to prove, characterize and assess (bio)transformation processes of chlorinated compounds in contaminated aquifers (Meckenstock et al., 2004; Kuntze et al., 2020). This technique relies on the fact that during (bio)transformation processes, the bonds of the contaminant containing the lighter isotopes are broken faster, causing the remaining contaminant to be enriched in the heavier isotopes compared to the original isotopic composition (Hunkeler and Bernasconi, 2010). This effect can be measured by quantifying the abundance ratio of specific stable isotopes (e.g. $^{13}C/^{12}C$, $^{37}Cl/^{35}Cl$) in contaminant molecules in relation to an international standard. Stable isotope analysis of a single element can be applied to assess a reaction mechanism. Also, a quantitative estimation of the extent of contaminant transformation in the field is possible when the isotope fractionation (ε) for a given compound is previously determined in field-derived microcosms in laboratory experiments (Aelion et al., 2010). Dual-element isotope analysis, however, enables a better characterization of a reaction mechanism and, in contrast to single element isotope fractionation analysis, the proportion of changes in isotope ratios of both elements relative to each other (e.g., slope $\Lambda_{C\text{-}Cl} = \Delta\delta^{13}C/\Delta\delta^{37}Cl$) is largely unaffected by nondegradative processes (Ojeda et al., 2020; Nijenhuis and Richnow, 2016).

In previous laboratory studies, different values of carbon isotopic fractionation (ε_C) for 1,2-DCP anaerobic dechlorination were reported for *Dehalogenimonas alkenigignens* strain BRE15 M (−15.0 ± 0.7‰) (Martín-González et al., 2015) and *Dehalococcoides mccartyi* strains RC and KS (−10.8 ± 0.9 and −11.3 ± 0.8‰, respectively) (Fletcher et al., 2009) even though both genera harbored the same functional 1,2-DCP-to-propene RdhA (DcpA). Recently, the first value of carbon isotopic fractionation for 1,2,3-TCP anaerobic dechlorination to allyl chloride has been reported in an enrichment culture that did not contain *Dehalogenimonas* (−5.2 ± 0.1‰) (Ning et al. 2022; Zhang et al., 2023). To date, multi-element isotope fractionation associated with 1,2-DCP and 1,2,3-TCP is missing and, thus, characterization of reaction mechanisms is not straightforward. For both compounds, dechlorination is expected to occur by dihaloelimination either *stepwise* (i.e., single C–Cl bond cleavage at the first reaction step) or *concertedly* (i.e., the two C–Cl bonds are broken simultaneously), or by nucleophilic substitution S_N2 reaction followed by a concerted syn-elimination (Elsner and Hofstetter, 2011; Torralba-Sanchez et al., 2020) (Fig. 1).

In recent multi-element (C–Cl and C–Br) studies transforming 1,2-dichloroethane (1,2-DCA) and 1,2-dibromoethane (1,2-DBA) by *Dehalogenimonas* and *Dehalococcoides*-containing cultures, distinct dual isotope slopes were observed for these two bacterial cultures (Palau et al., 2017; Franke et al., 2017, 2020), and finally interpreted as different reaction mechanisms (nucleophilic substitution S_N2 vs. dihaloelimination) (Heckel and Elsner, 2022; Palau et al., 2023). This was probably associated with different RdhAs, and, as observed for chlorinated ethenes (Lihl et al., 2019), pre-cultivation/substrate adaptation must have selected for reductive dehalogenases with different

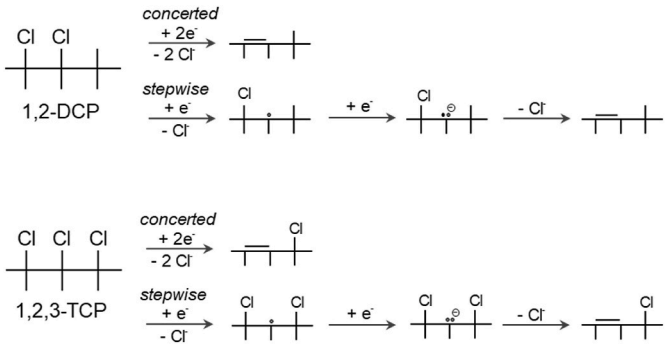

Fig. 1. Dichloroelimination pathways of 1,2-DCP and 1,2,3-TCP.

mechanistic motifs even within the same species. This illustrates a diversity of biochemical reaction mechanisms manifested even within the same class of enzymes (RdhAs). Since such evidences for diversity of mechanisms within RdhAs are missing for chlorinated propanes due to the lack of dual-isotope studies, the main goal of this study was the characterization of the reaction mechanisms associated with the dechlorination of 1,2-DCP and 1,2,3-TCP in *Dehalogenimonas alkenigignens* strain BRE15 M previously growing on the same substrate (1, 2-DCP) using dual element (C/Cl) CSIA. In addition, a secondary goal of this study was to investigate the substrate specificity of the RdhA expressed in strain BRE15 M grown with 1,2-DCP, since evidence that some RdhAs expressed in cultures enriched with a single substrate have the potential to dehalogenate multiple compounds is relatively scarce in the literature and it is mostly focused on the genus *Dehalococcoides* (Franke et al., 2020; Magnuson et al., 2000).

2. Material and methods

2.1. Dehalogenimonas-containing culture

This culture derived initially from sediments obtained from the Besòs River (Spain) and, after an enrichment process, it was mostly composed of bacteria belonging to the genus *Dehalogenimonas* (~70%) (Palau et al., 2023). The genome of this strain has been recently sequenced and annotated, and it was denominated *Dehalogenimonas alkenigignens* strain BRE15 M (Trueba-Santiso et al., 2021).

The culture used in this study was grown with 1,2-DCP as only halogenated substrate for more than five years, and it was maintained in our laboratory as described elsewhere (Martín-González et al., 2015; Trueba-Santiso et al., 2021). Briefly, the culture was grown in 100 mL glass serum bottles with 65 mL of anaerobic bicarbonate-buffered medium (pH = 7), reduced with $Na_2S \times 9H_2O$ and L-cysteine (0.2 mM each) amended with 5 mM sodium acetate as carbon source and gassed with N_2/CO_2 (4:1, v/v, 0.2 bar overpressure) and H_2 (added to an overpressure at 0.4 bar). All microcosms were cultivated under static conditions in the dark at 25 °C in a thermostatized chamber. Cultures used in this study consumed at least 2 mM of 1,2-DCP when used as inoculum for the below-described experiments.

2.2. Dehalogenase activity assays

For testing the substrate range of the 1,2-DCP grown cultures, cell suspensions were harvested using 210 mL of the *Dehalogenimonas*-containing culture. The culture was nitrogen-bubbled for 10 min to remove all volatile compounds and then concentrated 10-fold by centrifugation cycles at 9000×*g* at 10 °C for 40 min under anoxic conditions by transferring the culture into the Falcon tubes and removing the supernatant inside an anoxic tent (Coy Laboratories, USA) in an atmosphere composed of N_2 and 1–3% H_2. The enzymatic assay was performed under anoxic and static conditions in 10 mL vials with a reaction mixture that contained 500 μL of the cell concentrate, 0.1 M potassium acetate (pH 5.6), 2 mM Ti(III) citrate, 4 mM methyl viologen and 200 μM of the corresponding halogenated compound [1,2-DCP, 1,2,3-TCP, 1,1,2-trichloroethane (1,1,2-TCA), 1,2-DCA, 1,2-DBA, 1,2-dibromopropane (1,2-DBP), 1,3-dichloropropane (1,3-DCP), 1,1,1-trichloroethane (1,1,1-TCA)]. The final volume of the reaction mixture was 2 mL. Each halogenated compound was tested by triplicate. To discard abiotic transformations, two parallel controls were included: i) reaction mixture with halogenated compounds but without cells, and ii) reaction mixture with cells but without halogenated compounds. All controls were performed in triplicate.

An additional dehalogenase activity assay was performed from gel slices of blue native polyacrylamide gel electrophoresis (BNE) loaded with proteins of the *Dehalogenimonas*-containing culture grown with 1,2-DCP, as described in Supplementary Materials and Methods.

In all the assays, concentrations of halogenated substrates and their corresponding transformation products were quantified 24 h after the start of the experiment to allow the phase-equilibrium to occur in the headspace for sampling.

2.3. Biodegradation experiments for CSIA

To study the isotopic fractionation during 1,2-DCP and 1,2,3-TCP degradation, two parallel time-course degradation experiments were performed following the dechlorination assays described above. In this case, 140 mL of the *Dehalogenimonas*-containing culture that consumed at least 2 mM of 1,2-DCP was nitrogen-bubbled for 10 min to remove all volatile compounds and then concentrated 4-fold by centrifugation cycles at 9000×*g* at 10 °C for 40 min under anoxic conditions. The experiments for each chlorinated propane consisted of 20 replicates in 20 mL glass vials containing on each vial 1 mL of the cell concentrate and the target compound at 200 μM into a total volume of the reaction mixture of 10 mL. At each time point of analysis (each 20–30 min), 100 μL aerobic 1 M NaOH were added to the vials (pH~12) in the 1,2-DCP experiment and 250 μL 70% HNO_3 in the 1,2,3-TCP experiment (pH~2) to stop the reactions and as preservation agents. We did not employ NaOH to stop the reaction in the vials containing 1,2,3-TCP because it transformed abiotically this compound (data not shown). Each experiment included two different controls containing: (i) reaction mixture without chloropropanes + preservation agent, and (ii) reaction mixture without inoculum + target chloropropane + preserving agent. Controls (i) indicated the presence of volatile compounds derived from the inoculum or the reaction mixture, and controls (ii) ("abiotic controls") the initial concentration of the substrates, possible abiotic transformations of the chloropropanes by the preserving agents, Ti(III) citrate or methyl viologen radical. Headspace samples were taken for measuring concentration and liquid samples were stored at 4 °C until further isotopic analysis.

2.4. Concentration analysis

All mentioned halogenated substrates and their corresponding transformation products were analyzed from headspace samples with a gas chromatograph (GC) model 6890 N (Agilent Technologies; Santa Clara, USA) equipped with an HP-5 column (30 m × 0.32 mm with 0.25 μm film thickness; Agilent Technologies) and a flame ionization detector (FID). Helium was used as the carrier gas (1.5 mL min^{-1}). The injector and detector temperatures were both set at 250 °C. After the injection of the sample (split ratio = 2:1), the initial oven temperature (40 °C), ramped at 10 °C min^{-1} to 50 °C, and then ramped at 20 °C min^{-1} to 120 °C. Target compounds were identified and quantified using external chemical standards. Nominal concentrations are expressed as μmol per L of liquid volume or in nanomoles (nmol). Chromatograms showing the retention time of 1,2-DCP, 1,2,3-TCP, propene and allyl chloride are shown in Fig. S1.

2.5. CSIA analysis

Carbon isotope analyses were performed by headspace solid phase microextraction (HS-SPME) coupled to gas chromatography isotope ratio mass spectrometry (GC-IRMS) as described previously (Martín-González et al., 2015). Samples were analyzed in duplicate, and correction was made using daily values of calibrated in-house standards. Chlorine isotope analysis was conducted by headspace extraction coupled to gas chromatography quadrupole mass spectrometry (GC-qMS). Ten injections of each sample were performed, and a two-point calibration approach was used with two in-house working standards, also injected ten times each. Isotope ratios are reported using the delta notation in per mil, $\delta^{h}E = (R_{sample}/R_{standard}) - 1$, where *E* is the considered element (C or Cl), *h* is the atomic mass of the heavy isotope (13 for C and 37 for Cl) and *R* is the isotope ratio of carbon ($^{13}C/^{12}C$) or chlorine ($^{37}Cl/^{35}Cl$). The analytical uncertainty 1σ was ±0.4 ‰ for

carbon and ±1.0 ‰ for chlorine isotope values. More details on the isotope analysis methods can be found in the Supplementary Materials and Methods.

2.6. Isotope data evaluation

The carbon and chlorine isotopic fractionations (ε_C and ε_{Cl}) associated with the dechlorination of 1,2-DCP and 1,2,3-TCP were evaluated using the linearized form of the Rayleigh equation (Eq. 1). The uncertainty in the ε values was determined from the 95% confidence interval (95% CI) of the linear regression in the double logarithmic Rayleigh plots.

$$\ln\left(\frac{R_t}{R_0}\right) = \varepsilon \bullet \ln(f) \quad (1)$$

Apparent kinetic isotope effects (AKIE) were determined to characterize the isotope effect of the atoms at the reactive position of the molecules. The calculation of AKIE followed Eq. (2), where *n* represents the total number of atoms of the considered element (E) in the target molecule, *x* denotes the number of atoms situated at the reactive site, and *z* stands for the number of atoms in intramolecular isotopic competition (Elsner et al., 2005). This equation assumes the absence of secondary isotope effects. For carbon, secondary isotope effects are usually insignificant.

$$AKIE_E = \frac{1}{1 + \left(\frac{n \bullet z}{x} \bullet \varepsilon\right)} \quad (2)$$

For the calculation and interpretation of AKIE values, a hypothesis about the assumed reaction mechanism is necessary. For 1,2-DCP dichloroelimination to propene by a *stepwise* reaction (i.e., single C–Cl bond cleavage at the first reaction step), $n_C = 3$, $x_C = z_C = 1$ and $n_{Cl} = 2$, $x_{Cl} = z_{Cl} = 2$, as there is no intramolecular competition for C (the three C atoms are not in equivalent position), but there is for Cl (the two Cl atoms are in equivalent position). The same calculation would be assumed for S_N2 reaction followed by a concerted syn-elimination as observed for 1,2-DCA (Heckel and Elsner, 2022). Assuming a *concerted* mechanism (i.e., the two C–Cl bonds are broken simultaneously), $n_C = 3$, $x_C = 2$, $z_C = 1$ and $n_{Cl} = x_{Cl} = 2$, $z_{Cl} = 1$, since there is no intramolecular competition between the atoms (both C–Cl bond are involved in the reaction). Similarly, the following values were used for 1,2,3-TCP dichloroelimination to allyl chloride: $n_C = 3$, $x_C = z_C = 1$ and $n_{Cl} = x_{Cl} = z_{Cl} = 3$ for a *stepwise* reaction, and n = 3, x = 2, z = 1 for both C and Cl for a *concerted* reaction. The uncertainty of AKIE values was estimated by error propagation.

Dual isotope slopes ($\Lambda_{C/Cl}$) were calculated by ordinary linear regression of the changes in $\delta^{13}C$ and $\delta^{37}Cl$ data in 2D-isotope plots (i.e., $\Lambda_{C/Cl} = \Delta\delta^{13}C/\Delta\delta^{37}Cl$). The uncertainty of $\Lambda_{C/Cl}$ was reported as the 95% CI, determining the uncertainty of $\Delta\delta$ values by error propagation. To get further mechanistic insights, obtained $\Lambda_{C\text{-}Cl}$ values were compared to those estimated by Eq. (3), which expresses the dual C–Cl isotope slope for a given substrate and reaction as a function of AKIE values (Elsner, 2010).

$$\Lambda_{C-Cl} = \frac{\Delta\delta^{13}C}{\Delta\delta^{37}Cl} \approx \frac{\varepsilon^C_{bulk}}{\varepsilon^{Cl}_{bulk}} \approx \frac{\left(\frac{x}{n}\right)_C}{\left(\frac{x}{n}\right)_{Cl}} \bullet \frac{AKIE_C - 1}{AKIE_{Cl} - 1} \bullet \frac{1 + AKIE_C \bullet (z_C - 1)}{1 + AKIE_{Cl} \bullet (z_{Cl} - 1)} \quad (3)$$

Statistical differences with previously reported values for the estimated isotope fractionation values (ε_{Cl} and ε_C) and dual isotope slopes ($\Lambda_{C/Cl}$) were assessed using statistical two-tailed z-score tests (Ojeda et al., 2019). Differences were considered statistically significant at the $\alpha = 0.05$ confidence level, except when noted.

2.7. qPCR

Liquid samples (3 mL) from the *Dehalogenimonas*-containing culture growing with either 1,2-DCP or 1,2,3-TCP were centrifuged at 9000 g for 20 min. The supernatant was removed and the DNA was extracted using a NucleoSpin Tissue kit (Machery Nagel) following the manufacturer instructions and eluted in a final volume of 50 μL. The qPCR analyses were performed with a Lightcycler 480 instrument (LC480; Roche) using a set of primers (Dhgm478F and Dhgm536R) and a TaqMan probe (Dhgm500Probe) previously described to target *Dehalogenimonas* 16S rRNA genes (Yang et al., 2017). The qPCR run conditions and the standard curve for quantifying the gene copies obtained in the assays were performed as described elsewhere (Fernández-Verdejo et al., 2021). The 16S gene copies per mL of culture sample were determined from the standard curve (Fig. S2).

3. Results and discussion

3.1. Growth and substrate specificity of dehalogenimonas

The range of halogenated compounds transformed via dihaloelimination for this *Dehalogenimonas*-containing culture *in vivo* was tested in a previous study and included 1,2-DCP, 1,2,3-TCP, 1,2-DBA, 1,2-DBP, 1,1,2-TCA and 1,2-DCA (Martín-González et al., 2015). In the present study, the qPCR assay using primers Dhgm478F and Dhgm536R showed that the dechlorination of 325 μM of 1,2-DCP to propene in 77 d and 350 μM of 1,2,3-TCP to allyl chloride in 295 d by this culture was accompanied by an increase of 2.5 and 6.8-fold the *Dehalogenimonas* 16S rRNA gene copies, respectively, indicating that dechlorination reactions were coupled to growth (Fig. S3).

Interestingly, in the enzymatic assays with cell suspensions of this culture grown on 1,2-DCP and using methyl viologen as artificial electron donor, 1,2-DBA (97 ± 3%), 1,2,3-TCP (74 ± 1%), 1,1,2-TCA (69 ± 4%) and 1,2-DBP (62 ± 4%) were transformed at higher extent than 1,2-DCP (33 ± 3%) after 24 h (Fig. 2). As observed for analogue compounds that differ in the halogen substituent (i.e. 1,2-DCA/1,2-DBA and 1,2-DCP/1,2-DBP), the dehalogenating activity increased with the decreasing electronegativity and decreasing partial negative charge from the chlorine to the bromine substituents. Previous studies with the tetrachloroethene RdhA (PceA) of *S. multivorans* also showed the same dehalogenation pattern with analogous halogenated phenols (Kunze et al., 2017). The presence of a higher number of chlorine atoms in 1,2,3-TCP compared to 1,2-DCP increases the electronegativity of the former, which is, therefore, more susceptible to reduction. We know that our culture transformed 1,2-DCA to ethene *in vivo* (Palau et al., 2017), but the low observable degradation (14 ± 6%) in the enzymatic assays indicated that the 1,2-DCA-dechlorinating RdhA is not probably expressed during growth on 1,2-DCP. The lack of degradation of 1,1,1-TCA and 1,3-DCP was expected because strain BRE15 M only dihaloeliminates alkanes with vicinal halogenated carbon atoms.

The proteome of the cells used as inoculum in these enzymatic assays, published in a previous study (Trueba-Santiso et al., 2021), is summarized in Fig. S4. The inventory of RdhA proteins produced for strain BRE15 M showed that the most abundantly expressed was an ortholog of the 1,2-DCP-dechlorinating RdhA DcpA. To study in more detail whether 1,2,3-TCP was degraded by the same set of RdhAs expressed during growth with 1,2-DCP, in this study we conducted a dechlorination enzymatic assay using gel slices of blue native polyacrylamide gel electrophoresis (BNE) loaded with proteins of the *Dehalogenimonas*-containing culture grown with 1,2-DCP. In a previous study, we showed that after BNE separation of crude extracts of strain BRE15 M grown with 1,2-DCP, the dechlorinating activity of 1,2-DCP was mostly constrained to a gel fragment between 146 and 480 KDa when protein membrane samples were solubilized with 1% (w/v) DDM (Trueba-Santiso et al., 2021). Here, we followed the same procedure, but two lanes were tested for 1,2-DCP dechlorinating activity and two

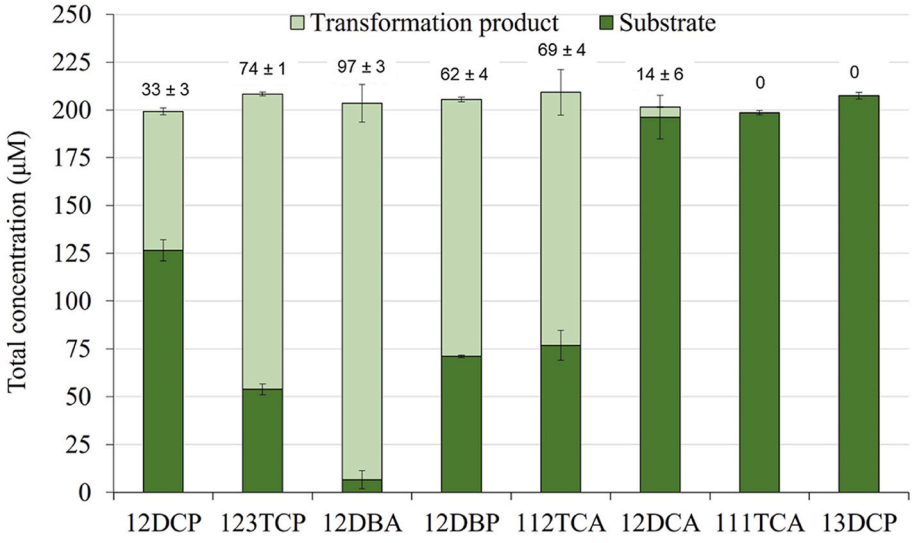

Fig. 2. Concentration of substrates and transformation products present in the enzymatic assays with cell suspensions of the culture containing strain BRE15 M after 24 h. Substrate transformation (% ± standard deviation) is shown on the top of each bar. Assays were performed in triplicate per each halogenated compound, with initial concentrations of 200 μM. The dihaloelimination reactions were: 1,2-DCP→propene; 1,2,3-TCP→allyl chloride; 1,2-dibromoethane (1,2-DBA)→ethene; 1,2-dibromopropane (1,2-DBP)→propene; 1,1,2-trichloroethane (1,1,2-TCA)→vinyl chloride; 1,2-dichloroethane (1,2-DCA)→ethene. No dechlorination was observed for 1,1,1-trichloroethane (1,1,1-TCA) and 1,3-dichloropropane (1,3-DCP).

parallel lanes were tested against 1,2,3-TCP. Each lane was cut into six slices and the enzymatic assays showed that dechlorination activity of 1,2-DCP and 1,2,3-TCP was again constrained to gel regions 3 and 4 (Fig. S5), which corresponded to 146–242 kDa and 242–480 kDa, respectively. Notably, the highest dechlorinating activity was found in both cases in the molecular mass range between 146 and 242 kDa, which coincides with the region with the highest abundance of the two unique RdhAs detected in the abovementioned study, a DcpA ortholog and a RdhA encoded adjacently to the DcpA ortholog (Trueba-Santiso et al., 2021). The colocalization of the dechlorinating activity of 1,2-DCP and 1,2,3-TCP strongly suggests that DcpA is responsible for the dihaloelimination of 1,2,3-TCP. This is in accordance with a previous study that detected *dcp*A gene transcripts in cultures of *Dehalogenimonas lykanthroporepellens* BL-DC-9T actively dechlorinating 1,2-DCP and 1,2,3-TCP, together with the simultaneous transcription of multiple additional *rdh* genes (Mukherjee et al., 2014).

3.2. Concentration and isotope patterns

3.2.1. Dechlorination of 1,2-DCP and 1,2,3-TCP by strain BRE15 M

Time-course degradation experiments of 1,2-DCP and 1,2,3-TCP were performed with cell suspensions of the *Dehalogenimonas*-containing culture. Dechlorination of ~200 μM 1,2-DCP proceeded rapidly, reaching 81 μM after 220 min, and producing propene as metabolite (Fig. 3A). Similarly, parallel enzymatic assays with the same inoculum but spiked with ~200 μM 1,2,3-TCP led to the production of allyl chloride (Fig. 3B). In both cases, the sum of moles of the parent compound and the metabolite (allyl chloride or propene) during dechlorination was within ±10% of the initial amount of 1,2-DCP and 1,2,3-TCP added at the beginning of the experiment, respectively, indicating quantitative conversion of 1,2,3-TCP and 1,2-DCP to allyl chloride and propene, respectively. The content of 1,2-DCP and 1,2,3-TCP in the abiotic controls remained at the initial concentration along the experiments (234 ± 5 μM, n = 5, for 1,2-DCP; 189 ± 13 μM, n = 4, for 1,2,3-TCP), whereas both chloropropanes were below detection limit along the controls (i). These results discarded the occurrence of abiotic transformation or the presence of other volatile compounds derived from the inoculum or the reaction mixture.

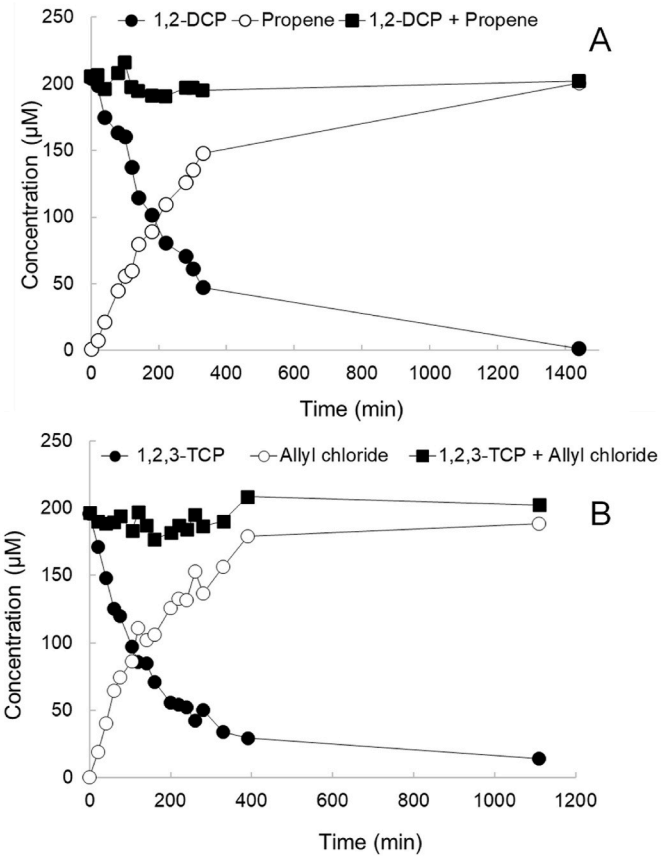

Fig. 3. Time-course degradation of 1,2-DCP and 1,2,3-TCP during enzymatic assays with cell suspensions of the *Dehalogenimonas*-containing culture. Each symbol represents the concentration of one replicate after stopping the reaction with the corresponding preservation agent.

3.2.2. Carbon and chlorine isotope fractionation patterns during 1,2-DCP and 1,2,3-TCP dechlorination

The $\delta^{13}C$ of 1,2-DCP and 1,2,3-TCP remained constant in the abiotic

controls, with a total average of −28.9 ± 0.5‰ and −27.1 ± 0.3‰, respectively. In the inoculated experiments, the $\delta^{13}C$ values showed a trend toward more positive values as dechlorination occurred, reflecting an enrichment of the heavy isotope (^{13}C). Thus, 1,2-DCP and 1,2,3-TCP reached a $\delta^{13}C$ value up to −7.3‰ and −17.0‰ when 79% and 92% of 1,2-DCP and 1,2,3-TCP were degraded, respectively. For both compounds, the changes in $\delta^{13}C$ values were well described by a Rayleigh equation (r^2_{12DCP} = 0.992, r^2_{123TCP} = 0.977), with resulting isotopic fractionations of $\varepsilon_{C,\ 12DCP} = -13.6 \pm 1.4$ ‰ and $\varepsilon_{C,\ 123TCP} = -3.8 \pm 0.6$ ‰ (Fig. 4A–B). This $\varepsilon_{C,\ 12DCP}$ value was statistically indistinguishable ($p > 0.01$) to the one previously obtained by combining cultures of this strain growing under methanogenic and non-methanogenic conditions (−15.0 ± 0.7‰, Martín-González et al., 2015), and thus, a combined value was determined (−14.8 ± 0.6‰, n = 27). This value differed slightly ($p < 0.05$) from the carbon isotope fractionation values obtained for *Dehalococcoides mccartyi* strains RC (−10.8 ± 0.9‰) and KS (−11.3 ± 0.8‰) (Fletcher et al., 2009) although the *dcpA* gene encoding 1, 2-DCP reductive dehalogenase was the same for both genera. Concerning 1,2,3-TCP, the $\varepsilon_{C,\ 123TCP}$ obtained in our study differed notably ($p < 0.01$) from that obtained by Zhang et al. (2023) with an anaerobic mixed enrichment culture that also transformed 1,2,3-TCP to allyl chloride (−5.2 ± 0.1‰). In this case, *Dehalogenimonas* was not detected in the culture (*Ning et al. 2022*) so the reasons of the deviation in the obtained ε_C values might be the involvement of other bacteria.

Regarding chlorine isotope results, the $\delta^{37}Cl$ of 1,2-DCP and 1,2,3-TCP in the abiotic controls did not change significantly during the experiment ($\delta^{37}Cl_{12DCP} = +0.7 \pm 0.7$‰, $\delta^{37}Cl_{123TCP} = -2.5 \pm 0.4$‰), while a significant enrichment in the heavy isotope (^{37}Cl) following a Rayleigh trend was observed during the dechlorination of 1,2-DCP ($\varepsilon_{Cl,\ 12DCP} = -27.4 \pm 5.2$‰). Compared to 1,2-DCP, a much weaker chlorine isotope fractionation was observed for 1,2,3-TCP ($\varepsilon_{Cl,\ 123TCP} = -0.8 \pm 0.5$‰), but still statistically significant ($p > 0.05$) (Fig. 4C–D). Compound-specific chlorine isotope analysis of both chloropropanes was performed for the first time, so then comparison of the obtained ε_{Cl} values with previous studies is not possible.

3.3. Insight into 1,2-DCP and 1,2,3-TCP dechlorination mechanisms

Table 1 summarizes obtained and previously reported isotope data for dichloroelimination and S_N2 reactions of chlorinated ethanes and propanes. Combining the isotope ratios of carbon and chlorine of 1,2-DCP and 1,2,3-TCP leads to a dual element isotope plot with linear trends (r^2_{12DCP} = 0.965, r^2_{123TCP} = 0.933) (Fig. 5). Different carbon and chlorine isotope fractionation patterns were observed, resulting in different dual C–Cl isotope slopes ($\Lambda_{123TCP} = 4 \pm 2$ and $\Lambda_{12DCP} = 0.5 \pm 0.1$). However, since direct comparison of dual C–Cl isotope slopes can not be made between different substrates (also due to different number of chlorines) and this is the first study including both carbon and chlorine isotope data for chloropropanes degradation, comparison of $\Lambda_{C\text{-}Cl}$ for different reactions and microbial strains is not possible, making a meaningful mechanistic comparison difficult. To improve this situation and to achieve a more in-depth understanding, further comparison is based on the isotope effect of the atoms at the reactive position of the molecules.

AKIEs were calculated for assessing reaction mechanisms (see results assuming *stepwise* (or S_N2) and *concerted* reactions in Table 1). $AKIE_C$ values for both chloropropanes are in accordance to those previously described for the same *Dehalogenimonas alkenigignens* strain BRE15 M (1,2-DCP, Martín-González et al., 2015), for *Dehalococcoides mccartyi* strains RC and KS (1,2-DCP, Fletcher et al., 2009) and for an enrichment culture (1,2,3-TCP, Zhang et al., 2023) (Table 1). All these values are below the theoretical Streitwieser limit for C–Cl bond breakage, $AKIE_C$ = 1.057 (Elsner et al., 2005), making them not conclusive to demonstrate the *stepwise* or *concerted* dichloroelimination reaction neither S_N2 mechanism of 1,2-DCP and 1,2,3-TCP. Nevertheless, since obtained $AKIE_C$ values for *stepwise* 1,2-DCP dichloroelimination are too close to those associated to abiotic *stepwise* dihaloelimination (1.04–1.05 for 1, 2-DCA, Vanstone et al., 2008; and 1.028–1.033 for 1,1,1-TCA, Elsner et al., 2007) which are often considered closest to the intrinsic isotope effects (Sherwood Lollar et al., 2010), a *concerted* mechanism involving two C–Cl bonds in the initial transformation step is more likely taking into account typical isotopic-masking effects occurring in bacteria-dependent reactions (e.g., non-fractionating binding of the substrate to the enzyme preceding bond cleavage). 1,2,3-TCP dichloroelimination has also previously assumed by computational chemistry calculations to be presumably a *concerted* reaction (Zhang et al., 2023). Further isotopic fractionation data from chloropropanes abiotic dihaloelimination experiments are required for confirming this hypothesis.

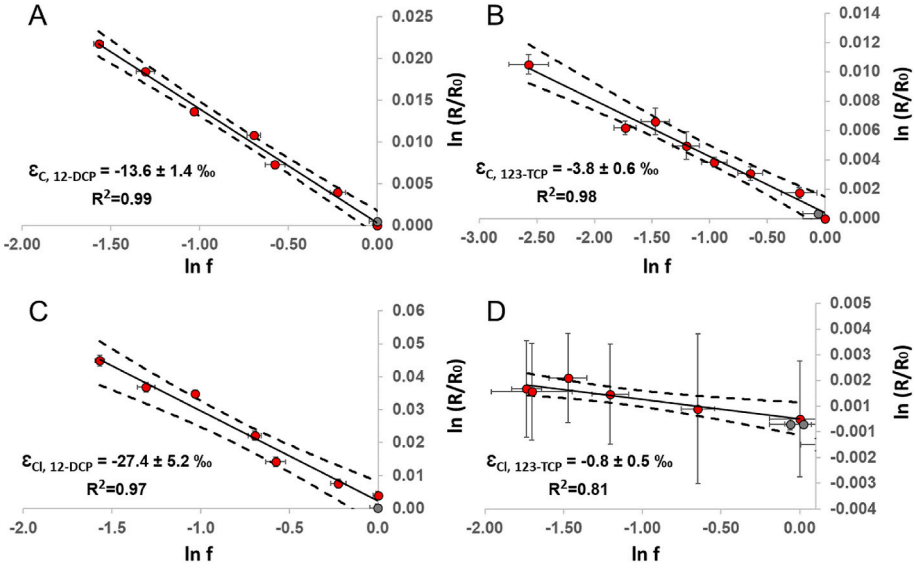

Fig. 4. Rayleigh plots for carbon (A, B) and chlorine (C, D) isotopic fractionation during 1,2-DCP (left panels) and 1,2,3-TCP (right panels) dechlorination in the enzymatic assays by the *Dehalogenimonas*-containing culture. Red circles represent time-course experiments with dehalogenase enzymatic assays, whereas grey circles stand to the abiotic controls. Doted lines correspond to the 95% confidence intervals of regression parameters. Error bars display the uncertainty calculated by error propagation including uncertainties in concentration and isotope measurements. Some error bars are smaller than the symbols. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Comparison of ε, $\Lambda_{C\text{-}Cl}$ and AKIE values for C and Cl isotopes for dechlorination of chlorinated propanes and ethanes assuming *stepwise* or *concerted* dichloroelimination reactions (except for hydrogenolysis for 1,1-DCA and 1,1,1-TCA dechlorination by ACT-3 culture, Sherwood Lollar et al., 2010; Phillips, 2021). n.m. not measured; n.a. not applicable; n.d. not determined; † The authors calculated AKIEs on the hypothesis of *stepwise* dichloroelimination but after Heckel and Elsner (2022), a S_N2 reaction was deduced, for which the same calculation applies. *The authors calculated $AKIE_{Cl}$ on the basis of the initial $\delta^{37}Cl$ signatures of reactants and the $\delta^{37}Cl$ value of the polychlorinated organic products after full conversion (Hofstetter et al., 2007); 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA), pentachloroethane (PCA), hexachloroethane (HCA).

Compound	Degradation experiment	εC (‰)	$AKIE_C$		εCl (‰)	$AKIE_{Cl}$		$\Lambda_{C\text{-}Cl}$	Reference
			Stepwise or S_N2	Concerted		Stepwise or S_N2	Concerted		
1,2-DCP	*Dehalogenimonas*-containing culture (strain BRE15 M)	−13.6 ± 1.4	1.043 ± 0.005	1.021 ± 0.002	−27.4 ± 5.2	1.06 ± 0.01	1.028 ± 0.006	0.5 ± 0.1	**this study**
1,2-DCP	*Dehalogenimonas alkenigignens* strain BRE15 M	−15.0 ± 0.7	1.045 ± 0.002	1.023 ± 0.001	n.m.			n.m.	Martín-González et al. (2015)
1,2-DCP	*Dehalococcoides mccartyi* strain RC	−10.8 ± 0.9	1.033 ± 0.003	1.016 ± 0.001	n.m.			n.m.	Fletcher et al. (2009)
1,2-DCP	*Dehalococcoides mccartyi* strain KS	−11.3 ± 0.8	1.033 ± 0.003	1.017 ± 0.001	n.m.			n.m.	Fletcher et al. (2009)
1,2,3-TCP	*Dehalogenimonas*-containing culture (strain BRE15 M)	−3.8 ± 0.6	1.012 ± 0.002	1.006 ± 0.001	−0.8 ± 0.5	1.002 ± 0.002	1.001 ± 0.001	4 ± 2	**this study**
1,2,3-TCP	Enrichment culture	−5.2 ± 0.1	1.016 ± 0.0003	1.008 ± 0.0002	n.m.			n.m.	Zhang et al. (2023)
1,2-DCA	*Dehalococcoides mccartyi* strain BTF08	−28.4 ± 3.7	1.059 ± 0.008†	1.029	−4.6 ± 0.7	1.009 ± 0.001†	1.005	6.9 ± 1.2	Franke et al. (2017)
1,2-DCA	*Dehalococcoides mccartyi* strain 195	−30.9 ± 3.6	1.066 ± 0.008†	1.031	−4.2 ± 0.5	1.009 ± 0.001†	1.004	7.1 ± 0.2	Franke et al. (2017)
1,2-DCA	*Dehalococcoides mccartyi* strain 195	−29.0 ± 3.0	1.062	1.030	n.m.			n.m.	Schmidt et al. (2014)
1,2-DCA	*Dehalococcoides mccartyi* strain BTF08	−30.8 ± 1.3	1.066	1.033	n.m.			n.m.	Schmidt et al. (2014)
1,2-DCA	*Dehalococcoides*-containing culture	−33.0 ± 0.4	1.071 ± 0.001†	1.034 ± 0.0004	−5.1 ± 0.1	1.010 ± 0.0002†	1.005 ± 0.0001	6.8 ± 0.2	Palau et al. (2017)
1,2-DCA	*Dehalogenimonas*-containing culture (strain BRE15 M)	−23 ± 2	1.048 ± 0.004	1.024 ± 0.003	−12.0 ± 0.8	1.025 ± 0.002	1.012 ± 0.001	1.9 ± 0.02	Palau et al. (2017)
1,2-DCA	*Dehalococcoides mccartyi* strain BTF08 (1,2-DCA used for cultivation of cells)	−27.5 ± 4.1	1.129 ± 0.021†	1.058 ± 0.018	−5.3 ± 0.6	1.023 ± 0.003†	1.009 ± 0.003	5.3 ± 0.6	Franke et al. (2020)
1,2-DCA	*Dehalococcoides mccartyi* strain BTF08 (cDCE used for cultivation of cells)	−18.5 ± 1.4	1.086 ± 0.024	1.038 ± 0.022	−4.5 ± 1.6	1.018 ± 0.005	1.009 ± 0.003	2.0 ± 0.5	Franke et al. (2020)
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 vitamin B12	−32.8 ± 1.7	1.06	–	−5.1 ± 0.2	1.01	–	6.4 ± 0.2	Heckel and Elsner (2022)
1,2-DCA	Abiotic by Zn°	−29.7 ± 1.5	1.06–1.07	1.03	n.m.			n.m.	Vanstone et al. (2008)
1,1-DCA	*Dehalobacter*-containing culture ACT-3 (whole cell)	−10.5 ± 0.6	1.021 ± 0.002	n.a	n.m.			n.m.	Sherwood Lollar et al. (2010)
1,1-DCA	*Dehalobacter*-containing culture ACT-3 (cell-free extracts)	−7.9 ± 0.9	1.016 ± 0.002	n.a	n.m.			n.m.	Sherwood Lollar et al. (2010)
1,1-DCA	*Dehalobacter*-containing culture ACT-3	−8.9 ± 1.0	1.018 ± 0.0022	n.a	−4.3 ± 0.5	1.009 ± 0.0008	n.a	2.1 ± 0.2	Phillips (2021)
1,1-DCA	Abiotic by Zn°	−19.2 ± 1.8	1.04–1.05	n.a	n.m.			n.m.	Vanstone et al. (2008)
1,1,2-TCA	*Dehalogenimonas*-containing culture	−6.9 ± 0.4	1.014 ± 0.001	1.007 ± 0.0004	−2.7 ± 0.3	1.008 ± 0.001	1.004 ± 0.0005	2.5 ± 0.2	Rosell et al. (2019)
1,1,2-TCA	Anoxic microcosms	−2.0 ± 0.2	1.004	1.002	n.m.			n.m.	Hunkeler et al. (2002)
1,1,2-TCA	Carbon-amended ZVI 20% (w/w)	−14.6 ± 0.7 to −0.7 ± 0.1	1.030 to 1.001	1.015 to 1.0007	n.m.			n.m.	Patterson et al. (2016)
1,1,2-TCA	Abiotic by Fe°	−12 ± 5	1.025	1.012	n.m.			n.m.	Patterson et al. (2016)
1,1,1-TCA	*Dehalobacter*-containing culture ACT-3 (whole cell)	−1.8 ± 0.3	1.004 ± 0.0006	n.a	n.m.			n.m.	Sherwood Lollar et al. (2010)
1,1,1-TCA	*Dehalobacter*-containing culture ACT-3 (cell-free extracts)	−0.8 ± 0.3	1.002 ± 0.0006	n.a	n.m.			n.m.	Sherwood Lollar et al. (2010)
1,1,1-TCA	*Dehalobacter*-containing culture ACT-3	−5.8 ± 0.8	1.012 ± 0.0017	n.a	−2.8 ± 0.3	1.008 ± 0.0008	n.a	2.1 ± 0.1	Phillips (2021)
1,1,1-TCA	Abiotic by Cr(II), Fe° and Cu-plated iron	−13.6 ± 0.5 to −15.8 ± 0.6	1.028 ± 0.001 to 1.033 ± 0.001	n.a	n.m.			n.m.	Elsner et al. (2007)
1,1,1-TCA	Abiotic by Fe°	−7.8 ± 0.4	1.016 ± 0.0008	n.a	−5.2 ± 0.2	1.016 ± 0.0006	n.a	1.5 ± 0.1	Palau et al. (2014a)

(*continued on next page*)

Table 1 (*continued*)

Compound	Degradation experiment	εC (‰)	$AKIE_C$		εCl (‰)	$AKIE_{Cl}$		$\Lambda_{C\text{-}Cl}$	Reference
			Stepwise or S_N2	Concerted		Stepwise or S_N2	Concerted		
1,1,1-TCA	Abiotic by FeS formed by biotic processes	−10.3 to −14.0			n.m.			n.m.	Broholm et al. (2014)
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)
1,1,2,2-TeCA	Abiotic by Cr(II), Fe^0 and Cu-plated iron	−17.0 ± 0.6 to −19.3 ± 0.7	1.035 ± 0.001 to 1.040 ± 0.001	1.017 ± 0.001 to 1.020 ± 0.001	n.m.			n.m.	Elsner et al. (2007)
PCA	Abiotic by Cr(II)	−14.7 ± 0.6	1.030 ± 0.001	n.d.	n.m.	1.021 ± 0.002*	n.d.	n.m.	Hofstetter et al. (2007)
HCA	Abiotic by Cr(II)	−10.4 ± 0.5	1.021 ± 0.001	n.d.	n.m.	1.013 ± 0.002*	n.d.	n.m.	Hofstetter et al. (2007)

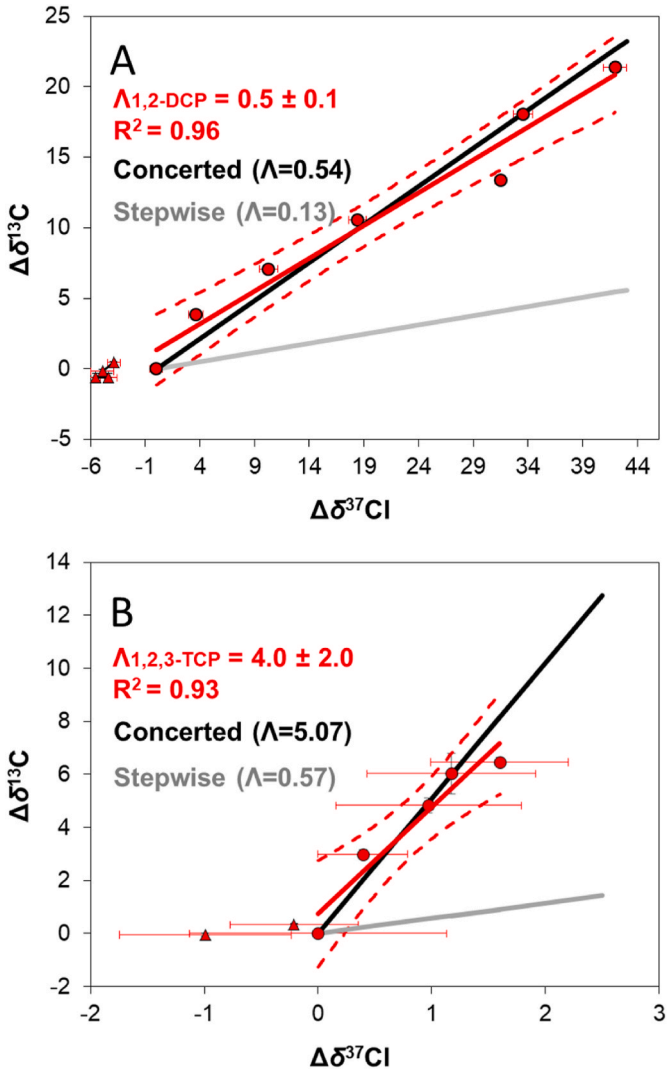

Fig. 5. Dual C–Cl isotope patterns during anaerobic biodegradation of 1,2-DCP (A) and 1,2,3-TCP (B) by the *Dehalogenimonas*-containing culture compared to their respective theoretical $\Lambda_{C\text{-}Cl}$ for *stepwise* (or S_N2) and *concerted* reactions as a function of the obtained AKIE values using Eq. (3). Triangles represent C–Cl isotope fractionation of abiotic controls. Dotted lines indicate the 95% confidence intervals of the linear regression and $\Lambda_{C\text{-}Cl}$ values (±95% C.I.) are given by the slope of the linear regressions. Error bars display the uncertainty calculated by error propagation.

Computational predictions would also be valuable for drawing definite conclusions.

As occurred with $AKIE_C$, obtained $AKIE_{Cl}$ values for 1,2,3-TCP dechlorination assuming *stepwise* (or S_N2) vs. *concerted* reaction mechanisms were also below the theoretical Streitwieser limit for C–Cl bond breakage, $AKIE_{Cl} = 1.013$ (Elsner et al., 2005), making both modes feasible, and, therefore, not allowing a broader mechanistic interpretation. Nevertheless, assuming *concerted* 1,2,3-TCP dichloroelimination, a $\Lambda_{C\text{-}Cl}$ value of 5.1 was estimated by Eq. (3), which is closer to the experimental value of 4 ± 2 (Fig. 5) than the estimated value of 0.57 assuming *stepwise* (or S_N2), suggesting a *concerted* dichloroelimination pathway, as it was postulated from $AKIE_C$.

In the case of 1,2-DCP, however, obtained $AKIE_{Cl}$ values are both above the Streitwieser limit for C–Cl bond breakage (1.013, Elsner et al., 2005) and the theoretical revised value (1.019, Paneth, 1992). High $AKIE_{Cl}$ values (1.013–1.021) have been already previously reported for dichloroelimination of polychlorinated ethanes (Hofstetter et al., 2007), but for abiotic reduction by Cr(II), not for biodegradation. As the *concerted*-assumed $AKIE_{Cl}$ value is above but closer to the theoretical one, it is more likely that the reaction proceeds via a *concerted* mechanism, involving both C–Cl bonds within the initial transformation step. In this way, $AKIE_{Cl}$ (1.028) > $AKIE_C$ (1.021) for 1,2-DCP dichloroelimination, which is not usual as primary isotope effects for carbon are generally expected to be higher than for chlorine (Elsner et al., 2005). However, unexpectedly high chlorine isotope effects were also observed for abiotic 1,1,1-TCA dichloroelimination by Fe^0 (Palau et al., 2014a) and 1,2-DCA aerobic biodegradation via C–Cl bond cleavage by S_N2 reaction ($AKIE_{Cl}$ (1.008) > $AKIE_C$ (1.007) (Palau et al., 2014b). In both cases, this phenomenon was explained by secondary chlorine isotope effects; this would point to S_N2 reaction as it would not work for 1,2-DCP when losing both chlorines simultaneously. However, assuming a *concerted* reaction, a $\Lambda_{C\text{-}Cl}$ value of 0.54 was estimated by Eq. (3), which is much more similar to the experimental value of 0.5 ± 0.1 (Fig. 5) than 0.13 for *stepwise* or S_N2 reactions, supporting again the assumption for a *concerted* reaction mechanism. Given that dechlorination of both chlorinated propanes seems to occur by a similar mechanism (*concerted* dichloroelimination), the observed differential chlorine isotope effect in 1,2-DCP contributes to the much more distinct dual C–Cl isotope slope compared to 1,2,3-TCP, but additional research is needed for finding the underlying reasons.

Finally, the relationship between AKIEs and involved RdhAs were further assessed to gain insights into RdhA behavior according to Phillips et al. (2022). Fig. 6 shows the differences in C and Cl AKIEs among different RdhAs previously reported to be involved in dechlorination of chlorinated ethanes, as well as the results of this study for chloropropanes. Although no clear correlation can be deduced from this graph, most of the RdhAs and abiotic reactions have a bit higher carbon than chlorine isotope fractionation keeping on a parallel line above the 1:1 ratio. Only, 1,1,1-TCA degradation by Fe^0 with above-mentioned

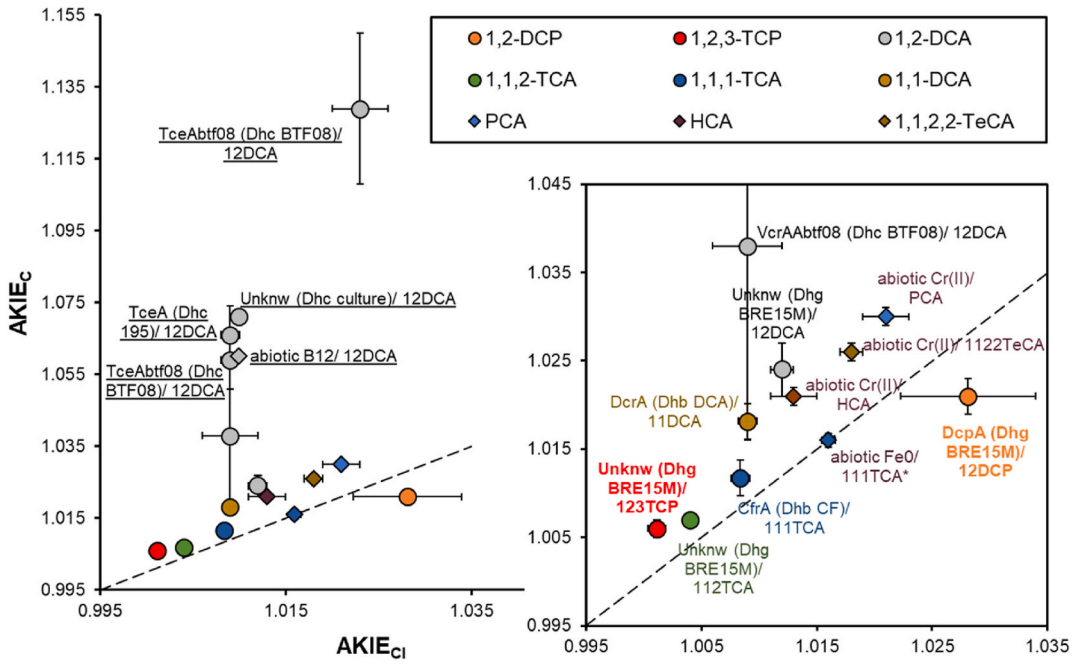

Fig. 6. $AKIE_C$ vs. $AKIE_{Cl}$ for reductive dechlorination of chlorinated propanes (this study) and ethanes by different reductive dehalogenases (RdhA). Each point is annotated showing RdhA name (strain)/substrate. Reference abiotic reactions are also shown (diamonds). Error bars show uncertainty in AKIEs, calculated in most cases by error propagation. In some cases, error bars are smaller than the symbols. The dotted line represents $AKIE_{Cl}/AKIE_C = 1$. The assumed S_N2 reaction for some 1,2-DCA cases (underlined) are shown in the left panel, whereas close-ups for the dichloroelimination reactions are shown in the right panel. *Concerted* reactions are assumed for 1,2-DCP, 1,2,3-TCP, 1,1,2-TCA and the remaining cases of 1,2-DCA. *Stepwise* reactions are assumed for 1,1-DCA, 1,1,1-TCA, 1,1,2,2-TeCA, PCA and HCA. Data and corresponding references are shown in Table 1. Dhg: *Dehalogenimonas*, Dhc: *Dehalococcoides*, Dhb: *Dehalobacter*, Unknw: unknown/uncharacterized RdhA, *potential secondary chlorine isotope effect.

presumably secondary chlorine isotope effect aligns with the 1:1 line (Palau et al., 2014a). Additionally, particularly higher $AKIE_C$ values were found when including S_N2 reactions for 1,2-DCA by *Dehalococcoides* strains (TceA, Franke et al., 2017, 2020, and uncharacterized enzyme, Palau et al., 2017), although they do not form a cohesive cluster with the abiotic vitamin B12 reaction (Heckel and Elsner 2022). Our results are clearly no part of this S_N2 trend, but 1,2,3-TCP and 1,2-DCP are extremely separated above and below the 1:1 line, respectively. The final distribution seems neither link to number of chlorines nor to hydrophobicity of the compounds.

The involvement of the RdhA DcpA was previously proved for 1,2-DCP dechlorination (Padilla-Crespo et al., 2014), and is the most abundantly expressed RdhA in *Dehalogenimonas alkenigignens* BRE15 M used in this study (Trueba-Santiso et al., 2021). The involvement of DcpA in 1,2,3-TCP dechlorination was also previously suggested (Mukherjee et al., 2014) and is additionally supported by the colocalization of the dechlorinating activity of 1,2-DCP and 1,2,3-TCP in the dehalogenase activity assays from BNE gel slices of this study. However, the different isotopic behavior for 1,2,3-TCP dechlorination compared to 1,2-DCP opens the possibility that a minor expressed additional dehalogenase, different than DcpA, might be responsible of 1,2,3-TCP dechlorination by *Dehalogenimonas alkenigignens* BRE15 M, since this strain expresses a suite of ten different RdhA enzymes (Fig. S4). Further research is required to confirm this hypothesis.

4. Conclusions

The occurrence of 1,2-DCP and 1,2,3-TCP in groundwaters is of concern due to their toxicity and relative persistence. Due to their relatively low Henry's Law constant, the removal of 1,2-DCP and 1,2,3-TCP is less effective when air stripping, air sparging, and soil vacuum extraction is applied in comparison with other chlorinated compounds (for instance trichloroethylene, vinyl chloride or chloroform). On the contrary, enhanced anaerobic bioremediation is an efficient alternative treatment that is also preferable because benign products are produced during dechlorinating reactions. Our findings that *Dehalogenimonas* cells grown with 1,2-DCP transformed 1,2,3-TCP in enzymatic assays using cell suspensions and slices of BNE gels, leave open the possibility that DcpA, which was previously identified as encoding a 1,2-DCP reductive dehalogenase, was also responsible for transformation of 1,2,3-TCP. This can be particularly interesting for the remediation of sites impacted with mixtures of 1,2-DCP and 1,2,3-TCP, which is a common scenario in groundwaters polluted with fumigants in agricultural zones. In contrast, the enzymatic assays indicate that Rdh involved in 1,2-DCA dechlorination is not likely expressed when 1,2-DCP is used as electron acceptor, so future studies are needed to identify the enzyme responsible for 1,2-DCA dechlorination by this strain.

Other chlorine isotopic fractionation values for anaerobic dechlorination of 1,2-DCP and 1,2,3-TCP are not available in the literature yet for comparison. Therefore, this is a very valuable information for future studies since knowledge of the compound- and reaction-specific fractionation is necessary to assess the degree of biodegradation using CSIA. The dechlorination of 1,2,3-TCP was associated with a small carbon and chlorine isotope effects, giving rise to a large dual element isotope slope. In contrast, dihaloelimination of 1,2-DCP was associated with pronounced isotope effects in both elements and unexpectedly higher in chlorine, producing a smaller dual element isotope slope. The single element kinetic isotope effects could not provide conclusive information about the reaction mechanism involved in these chlorinated propanes (*concerted* or *stepwise*); however, the dual-element approach could reduce interpretation bias due to isotope-masking effects overcoming this limitation and pointing to more likely *concerted* mechanism for both compounds. Taking this for granted, the observed isotopic differences must inevitably be linked to the RdhA. Our results suggest that DcpA is involved in the dechlorination of both substrates, but new possibilities emerge, such as the involvement of alternative RdhAs expressed at lower abundance. Further investigations on carbon and chlorine isotope fractionation with bacteria catalyzing alternative degradation pathways or

abiotic reactions will help us to understand the scope of our research in enabling their distinction in field studies.

CRediT authorship contribution statement

Alba Trueba-Santiso: Investigation, Methodology, Writing – review & editing. **Clara Torrentó:** Data curation, Investigation, Methodology, Writing – review & editing. **Jesica M. Soder-Walz:** Investigation, Methodology, Writing – review & editing. **David Fernández-Verdejo:** Investigation, Methodology, Writing – review & editing. **Mònica Rosell:** Writing – review & editing, Methodology, Investigation, Data curation. **Ernest Marco-Urrea:** Writing – original draft, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.142170.

References

Aelion, C.M., Höhener, P., Hunkeler, D., Aravena, R., 2010. Environmental Isotopes in Biodegradation and Bioremediation. CRC Press, Boca Ratón, London, New York.

Broholm, M.M., Hunkeler, D., Tuxen, N., Jeannottat, S., Scheutz, C., 2014. Stable carbon isotope analysis to distinguish biotic and abiotic degradation of 1,1,1-trichloroethane in groundwater sediments. Chemosphere 108, 265–273. https://doi.org/10.1016/j.chemosphere.2014.01.051.

Burow, K.R., Floyd, W.D., Landon, M.K., 2019. Factors affecting 1,2,3-trichloropropane contamination in groundwater in California. Sci. Total Environ. 672, 324–334. https://doi.org/10.1016/j.scitotenv.2019.03.420.

de Wildeman, S., Diekert, G., van Langenhove, H., Verstraete, W., 2003. Stereoselective microbial dehalorespiration with vicinal dichlorinated alkanes. Appl. Environ. Microbiol. 69, 5643–5647. https://doi.org/10.1128/AEM.69.9.5643-5647.2003.

Elsner, M., 2010. Stable isotope fractionation to investigate natural transformation mechanisms of organic contaminants: principles, prospects and limitations. J. Environ. Monit.: JEM (J. Electron Microsc.) 12, 2005–2031. https://doi.org/10.1039/C0EM00277A.

Elsner, M., Hofstetter, T.B., 2011. Current perspectives on the mechanisms of chlorohydrocarbon degradation in subsurface environments: insight from kinetics, product formation, probe molecules, and isotope fractionation. Aquatic Redox Chemistry 407–439. https://doi.org/10.1021/bk-2011-1071.ch019.

Elsner, M., Zwank, L., Hunkeler, D., Schwarzenbach, R.P., 2005. A new concept linking observable stable isotope fractionation to transformation pathways of organic pollutants. Environ. Sci. Technol. 39, 6896–6916. https://doi.org/10.1021/es0504587.

Elsner, M., Cwiertny, D.M., Roberts, A.L., Sherwood Lollar, B., 2007. 1,1,2,2-tetrachloroethane reactions with OH˙, Cr (II), granular iron, and a copper−iron bimetal: insights from product formation and associated carbon isotope fractionation. Environ. Sci. Technol. 41, 4111–4117. https://doi.org/10.1021/es063040x.

EPA, 2014. Technical Fact Sheet – 1,2,3-trichloropropane (TCP). U.S Environmental Protection Agency.

Fernández-Verdejo, D., Cortés, P., Blánquez, P., Marco-Urrea, E., Guisasola, A., 2021. Enhanced dechlorination of 1,2-dichloropropane to propene in a bioelectrochemical system mediated by *Dehalogenimonas*. J. Hazard Mater. 416, 126234 https://doi.org/10.1016/j.jhazmat.2021.126234.

Fletcher, K.E., Löffler, F.E., Richnow, H.H., Nijenhuis, I., 2009. Stable carbon isotope fractionation of 1,2-dichloropropane during dichloroelimination by Dhalococcoides populations. Environ. Sci. Technol. 43, 6915–6919. https://doi.org/10.1021/es900365x.

Franke, S., Lihl, C., Renpenning, J., Elsner, M., Nijenhuis, I., 2017. Triple-element compound-specific stable isotope analysis of 1, 2-dichloroethane for characterization of the underlying dehalogenation reaction in two *Dehalococcoides mccartyi* strains. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 93, fix137 https://doi.org/10.1093/femsec/fix137.

Franke, S., Seidel, K., Adrian, L., Nijenhuis, I., 2020. Dual element (C/Cl) isotope analysis indicates distinct mechanisms of reductive dehalogenation of chlorinated ethenes and dichloroethane in Dehalococcoides mccartyi strain BTF08 with defined reductive dehalogenase Inventories. Front. Microbiol. 11, 1507. https://doi.org/10.3389/fmicb.2020.01507.

Heckel, B., Elsner, M., 2022. Exploring mechanisms of biotic chlorinated alkane reduction: evidence of nucleophilic substitution (S_N2) with vitamin B12. Environ. Sci. Technol. 56, 6325. https://doi.org/10.1021/acs.est.1c06066.

Hofstetter, T.B., Reddy, C.M., Heraty, L.J., Berg, M., Sturchio, N.C., 2007. Carbon and chlorine isotope effects during abiotic reductive dechlorination of polychlorinated ethanes. Environ. Sci. Technol. 41, 4662–4668. https://doi.org/10.1021/es0704028.

Hunkeler, D., Bernasconi, S., 2010. Analysis of stable isotopes. In: Aelion, C.M., Höhener, P., Hunkeler, D., Aravena, R. (Eds.), Environmental Isotopes in Biodegradation and Bioremediation. CRC Press, Boca Ratón, London, New York, pp. 23–42.

Hunkeler, D., Aravena, R., Cox, E., 2002. Carbon isotopes as a tool to evaluate the origin and fate of vinyl chloride: laboratory experiments and modeling of isotope evolution. Environ. Sci. Technol. 36, 3378–3384. https://doi.org/10.1021/es011479d.

Key, T.A., Richmond, D.P., Bowman, K.S., Cho, Y., Chun, J., Costa, M.S., Rainey, F.A., Moe, W.M., 2016. Genome sequence of the organohalide- respiring *Dehalogenimonas alkenigignens* type strain (IP3-3 T). Standards in Genomic Sciences 1–11. https://doi.org/10.1186/s40793-016-0165-7.

Key, T.A., Bowman, K.S., Lee, I., Chun, J., Albuquerque, L., da Costa, M.S., Rainey, F.A., Moe, W.M., 2017. *Dehalogenimonas formicexedens* sp. nov., a chlorinated alkanerespiring bacterium isolated from contaminated groundwater. Int. J. Syst. Evol. Microbiol. 67, 1366–1373. https://doi.org/10.1099/ijsem.0.001819.

Kuntze, K., Eisenmann, H., Richnow, H.-H., Fischer, A., 2020. Compound-specific stable isotope analysis (CSIA) for evaluating degradation of organic pollutants: an overview of field case studies. In: Anaerobic Utilization of Hydrocarbons, Oils, and Lipids. Springer International Publishing, pp. 323–360.

Kunze, C., Bommer, M., Hagen, W.R., Uksa, M., Dobbek, H., Schubert, T., Diekert, G., 2017. Cobamide-mediated enzymatic reductive dehalogenation via long-range electron transfer. Nat. Commun. 8, 15858 https://doi.org/10.1038/ncomms15858.

Lihl, C., Douglas, L.M., Franke, S., Pérez-de-Mora, A., Meyer, A.H., Daubmeier, M., Edwards, E.A., Nijenhuis, I., Sherwood Lollar, B., Elsner, M., 2019. Mechanistic dichotomy in bacterial trichloroethene dechlorination revealed by carbon and chlorine isotope effects. Environ. Sci. Technol. 53, 4245–4254. https://doi.org/10.1021/acs.est.8b06643.

Magnuson, J.K., Romine, M.F., Burris, D.R., Kingsley, M.T., 2000. Trichloroethene reductive dehalogenase from Dehalococcoides ethenogenes: sequence of tceA and substrate range characterization. Appl. Environ. Microbiol. 66, 5141–5147. https://doi.org/10.1128/aem.66.12.5141-5147.2000.

Martín-González, L., Mortan, S.H., Rosell, M., Parladé, E., Martínez-Alonso, M., Gaju, N., Caminal, G., Adrian, L., Marco-Urrea, E., 2015. 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. 49, 8666–8674. https://doi.org/10.1021/acs.est.5b00929.

Meckenstock, R.U., Morasch, B., Griebler, C., Richnow, H.H., 2004. Stable isotope fractionation analysis as a tool to monitor biodegradation in contaminated aquifers. J. Contam. Hydrol. 75, 215–255. https://doi.org/10.1016/j.jconhyd.2004.06.003.

Moe, W.M., Yan, J., Nobre, M.F., da Costa, M.S., Rainey, F.A., 2009. *Dehalogenimonas lykanthroporepellens* gen. nov., sp. nov., a reductively dehalogenating bacterium isolated from chlorinated solvent-contaminated groundwater. Int. J. Syst. Evol. Microbiol. 59, 2692–2697. https://doi.org/10.1099/ijs.0.011502-0.

Molenda, O., Tang, S., Edwards, E.A., 2016. Complete genome sequence of *Dehalococcoides mccartyi* strain WBC-2, capable of anaerobic reductive dechlorination of vinyl chloride. Genome Announc. 4 https://doi.org/10.1128/genomeA.01375-16.

Mukherjee, K., Bowman, K.S., Rainey, F.A., Siddaramappa, S., Challacombe, J.F., Moe, W.M., 2014. *Dehalogenimonas lykanthroporepellens* BL-DC-9T simultaneously transcribes many rdhA genes during organohalide respiration with 1,2-DCA, 1,2-DCP, and 1,2,3-TCP as electron acceptors. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett. 354, 111–118. https://doi.org/10.1111/1574-6968.12434.

Nijenhuis, I., Richnow, H.H., 2016. Stable isotope fractionation concepts for characterizing biotransformation of organohalides. Curr. Opin. Biotechnol. 41, 108–113. https://doi.org/10.1016/j.copbio.2016.06.002.

Ning, Z., Zhang, M., Zhang, N., Guo, C., Hao, C., Zhang, S., Shi, C., Sheng, Y., Chen, Z., 2022. Metagenomic characterization of a novel enrichment culture responsible for dehalogenation of 1,2,3-trichloropropane to allyl chloride. J. Environ. Chem. Eng. 10, 108907 https://doi.org/10.1016/j.jece.2022.108907.

Ojeda, A.S., Phillips, E., Mancini, S.A., Lollar, B.S., 2019. Sources of uncertainty in biotransformation mechanistic interpretations and remediation studies using CSIA. Anal. Chem. 91, 9147–9153. https://doi.org/10.1021/acs.analchem.9b01756.

Ojeda, A.S., Phillips, E., Sherwood Lollar, B., 2020. Multi-element (C, H, Cl, Br) stable isotope fractionation as a tool to investigate transformation processes for halogenated hydrocarbons. Environmental Science: Process. Impacts 22, 567–582. https://doi.org/10.1039/C9EM00498J.

Padilla-Crespo, E., Yan, J., Swift, C., Wagner, D.D., Chourey, K., Hettich, R.L., Ritalahti, K.M., Löffler, F.E., 2014. Identification and environmental distribution of *dcpA*, which encodes the reductive dehalogenase catalyzing the dichloroelimination of 1,2-dichloropropane to propene in organohalide-respiring Chloroflexi. Appl. Environ. Microbiol. 80, 808–818. https://doi.org/10.1128/AEM.02927-13.

Palau, J., Shouakar-Stash, O., Hunkeler, D., 2014a. Carbon and chlorine isotope analysis to identify abiotic degradation pathways of 1, 1, 1-trichloroethane. Environ. Sci. Technol. 48, 14400–14408. https://doi.org/10.1021/es504252z.

Palau, J., Cretnik, S., Shouakar-Stash, O., Hoche, M., Elsner, M., Hunkeler, D., 2014b. C and Cl isotope fractionation of 1, 2-dichloroethane displays unique $\delta^{13}C/\delta^{37}Cl$ patterns for pathway identification and reveals surprising C–Cl bond involvement in microbial oxidation. Environ. Sci. Technol. 48, 9430–9437. https://doi.org/10.1021/es5031917.

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., 2017. 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. 51, 2685–2694. https://doi.org/10.1021/acs.est.6b04998.

Palau, J., Trueba-Santiso, A., Yu, R., Hatijah Mortan, S., Shouakar-Stash, O., Freedman, D.L., Wasmund, K., Hunkeler, D., Marco-Urrea, E., Rosell, M., 2023. Dual C–Br isotope fractionation indicates distinct reductive dehalogenation mechanisms of 1,2-dibromoethane in *Dehalococcoides*- and *Dehalogenimonas*-containing cultures. Environ. Sci. Technol. 57, 1949–1958. https://doi.org/10.1021/acs.est.2c07137.

Paneth, P., 1992. How to measure heavy atom isotope effects: general principles. In: Buncel, E., Saunders, W.H.J. (Eds.), Isotopes in Organic Chemistry. Elsevier, New York, p. 1992.

Patterson, B.M., Lee, M., Bastow, T.P., Wilson, J.T., Donn, M.J., Furness, A., Goodwin, B., Manefield, M., 2016. Concentration effects on biotic and abiotic processes in the removal of 1,1,2-trichloroethane and vinyl chloride using carbon-amended ZVI. J. Contam. Hydrol. 188, 1–11. https://doi.org/10.1016/j.jconhyd.2016.02.004.

Phillips, E., 2021. Use of compound-specific isotope analysis to investigate enzymatic reaction mechanisms. In: PhD Thesis. University of Toronto, p. 242.

Phillips, E., Bulka, O., Picott, K., Kümmel, S., Edwards, E.A., Nijenhuis, I., Gehre, M., Dworatzek, S., Webb, J., Sherwood Lollar, B., 2022. Investigation of active site amino acid influence on carbon and chlorine isotope fractionation during reductive dechlorination. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 98, fiac072. https://doi.org/10.1093/femsec/fiac072.

Ritalahti, K.M., Löffler, F.E., 2004. Populations implicated in anaerobic reductive dechlorination of 1,2-dichloropropane in highly enriched bacterial communities. Appl. Environ. Microbiol. 70, 4088–4095. https://doi.org/10.1128/AEM.70.7.4088-4095.2004.

Rosell, M., Palau, J., Mortan, S.H., Caminal, G., Soler, A., Shouakar-Stash, O., Marco-Urrea, E., 2019. Dual carbon-chlorine isotope fractionation during dichloroelimination of 1, 1, 2-trichloroethane by an enrichment culture containing *Dehalogenimonas* sp. Sci. Total Environ. 648, 422–429. https://doi.org/10.1016/j.scitotenv.2018.08.071.

Samin, G., Janssen, D.B., 2012. Transformation and biodegradation of 1,2,3-trichloropropane (TCP). Environ. Sci. Pollut. Control Ser. 19, 3067–3078. https://doi.org/10.1007/s11356-012-0859-3.

Schlötelburg, C., von Wintzingerode, C., Hauck, R., von Wintzingerode, F., Hegemann, W., Göbel, U.B., 2002. Microbial structure of an anaerobic bioreactor population that continuously dechlorinates 1,2-dichloropropane. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 39, 229–237. https://doi.org/10.1016/S0168-6496(02)00177-0.

Schmidt, M., Lege, S., Nijenhuis, I., 2014. Comparison of 1, 2-dichloroethane, dichloroethene and vinyl chloride carbon stable isotope fractionation during dechlorination by two *Dehalococcoides* strains. Water Res. 52, 146–154. https://doi.org/10.1016/j.watres.2013.12.042.

Sherwood Lollar, B., Hirschorn, S., Mundle, S.O., Grostern, A., Edwards, E.A., Lacrampe-Couloume, G., 2010. Insights into enzyme kinetics of chloroethane biodegradation using compound specific stable isotopes. Environ. Sci. Technol. 44, 7498–7503. https://doi.org/10.1021/es101330r.

Torralba-Sanchez, T.L., Bylaska, E.J., Salter-Blanc, A.J., Meisenheimer, D.E., Lyon, M.A., Tratnyek, P.G., 2020. Reduction of 1, 2, 3-trichloropropane (TCP): pathways and mechanisms from computational chemistry calculations. Environmental Science: Process. Impacts 22, 606–616. https://doi.org/10.1039/C9EM00557A.

Trueba-Santiso, A., Wasmund, K., Soder-Walz, J.M., Marco-Urrea, E., Adrian, L., 2021. Genome sequence, proteome profile, and identification of a multiprotein reductive dehalogenase complex in Dehalogenimonas alkenigignens strain BRE15M. J. Proteome Res. 20, 613–623. https://doi.org/10.1021/acs.jproteome.0c00569.

VanStone, N., Elsner, M., Lacrampe-Couloume, G., Mabury, S., Sherwood Lollar, B., 2008. Potential for identifying abiotic chloroalkane degradation mechanisms using carbon isotopic fractionation. Environ. Sci. Technol. 42, 126–132. https://doi.org/10.1021/es0711819.

Yan, J., Rash, B.A., Rainey, F.A., Moe, W.M., 2009. Isolation of novel bacteria within the Chloroflexi capable of reductive dechlorination of 1,2,3-trichloropropane. Environ. Microbiol. 11 (4), 833–843. https://doi.org/10.1111/j.1462-2920.2008.01804.x.

Yang, Y., Higgins, S.A., Yan, J., Şimşir, B., Chourey, K., Iyer, R., Hettich, R.L., Baldwin, B., Ogles, D.M., Löffler, F.E., 2017. Grape pomace compost harbors organohalide-respiring *Dehalogenimonas* species with novel reductive dehalogenase genes. ISME J. 12, 2767–2780. https://doi.org/10.1038/ismej.2017.127.

Zhang, M., Ning, Z., Guo, C., Shi, C., Zhang, S., Sheng, Y., Chen, Z., 2023. Using compound specific isotope analysis to decipher 1,2,3-trichloropropane-to-allyl chloride transformation by groundwater microbial communities. Environ. Pollut. 316, 120577 https://doi.org/10.1016/j.envpol.2022.120577.