Variable dual carbon-bromine stable isotope fractionation during enzyme-catalyzed reductive dehalogenation of brominated ethenes

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Abstract

The potential of compound-specific stable isotope analysis (CSIA) to characterize biotransformation of brominated organic compounds (BOCs) was assessed and compared to chlorinated analogues. *Sulfurospirillum multivorans* and *Desulfitobacterium hafniense* PCE-S catalyzed the dehalogenation of tribromoethene (TBE) to either vinyl bromide (VB) or ethene, respectively. Significantly lower isotope fractionation was observed for TBE dehalogenation by *S. multivorans* (ε_C = -1.3±0.2‰) compared to *D. hafniense* (ε_C = -7.7±1.5‰). However, higher fractionation was observed for dibromoethene (DBE) dehalogenation by *S. multivorans* (ε_C = -16.8±1.8‰ and -21.2±1.6‰ for *trans*- and *cis*-1,2- (DBE) respectively) compared to *D. hafniense* PCE-S (ε_C = -9.5±1.2‰ and -14.5±0.7‰ for *trans*-1,2-DBE and *cis*-1,2-DBE, respectively). Significant, but similar, bromine fractionation was observed for for *S. multivorans* (ε_{Br} = -0.53±0.15‰, -1.03±0.26‰, and -1.18±0.13‰ for *trans*-1,2-DBE, *cis*-1,2-DBE and TBE, respectively) and *D. hafniense* PCE-S (ε_{Br} = -0.97±0.28‰, -1.16±0.36‰, and -1.34±0.32‰ for *cis*-1,2-DBE, TBE and *trans*-1,2-DBE, respectively). Variable C-Br dual-element slopes were estimated at Λ (ε_C /ε_{Br}) = 1.03±0.2, 17.9±5.8, and 29.9±11.0 for *S. multivorans* debrominating TBE, *cis*-1,2-DBE and *trans*-1,2-DBE, respectively, and at 7.14±1.6, 8.27±3.7, and 8.92±2.4 for *D. hafniense* PCE-S debrominating *trans*-1,2-DBE, TBE and *cis*-1,2-DBE, respectively. A high variability in isotope fractionation, which was substrate property related, was observed for *S. multivorans* but not *D. hafniense*, similar as observed for chlorinated ethenes, and may be due to rate-limiting steps preceding the bond-cleavage or differences in the reaction mechanism. Overall, significant isotope fractionation was observed and, therefore, CSIA can be applied to monitor the fate of brominated ethenes in the environment. Isotope effects differences, however, are not systematically comparable to chlorinated ethenes.

Keywords: CSIA, reductive dehalogenation, brominated organic compounds

1. Introduction

Several chlorinated as well as brominated organic compounds (BOCs) are pervasive environmental contaminants (de Wit, 2002; Alaee, 2003); however, while biotransformation processes have been extensively investigated for chlorinated compounds (Bradley and Chapelle, 2010), very little is known regarding the environmental fate and transport of BOCs, such as the flame-retardants like polybrominated diphenyl ethers (PBDEs), and fumigants like ethylene dibromide (EDB) and methyl bromide (Waaijers and Parsons, 2016).

Microbial reductive dehalogenation is widely regarded as a key process in organohalide removal in environmental systems, and reductive dehalogenation of chloroethenes, for example, has been subject to considerable study (Smidt, 2004; Nijenhuis and Kuntze, 2016). Furthermore, validated concepts and approaches have been developed to address the fate of these chlorinated substances *in situ* (Bombach et al., 2010), but have yet to be developed and validated for their brominated analogues.

Compound-specific stable isotope analysis (CSIA) may be a suitable approach to monitor and characterize biodegradation of BOCs, as it is currently well developed for many chlorinated groundwater contaminants (for a review see (Hunkeler et al., 2008; Nijenhuis and Kuntze, 2016)). CSIA utilizes the relatively faster chemical reaction of molecules with a lighter isotope at the reactive position, resulting in an enrichment of heavy isotopes in the residual contaminant

63 pool (i.e. fractionation) and allowing for the calculation of reaction-specific enrichment factors
64 (ε), identifiers for detecting and monitoring *in situ* contaminant transformation (Hunkeler et al.,
65 2008).

66 Carbon isotope analysis has been extensively reported during microbial reductive dechlorination
67 of chloroethenes (see e.g. (Lee, 2007; Cichocka, 2008)), and dual-element carbon-chlorine
68 isotope analysis has also emerged for the same transformation process (Abe, 2009; Wiegert,
69 2013; Badin, 2014). For example, dual-element isotope analysis was described for reductive
70 dechlorination of chloroethenes by *Sulfurospirillum multivorans* and *Desulfitobacterium*
71 *hafniense* PCE-S (Cretnik, 2013; Renpenning, 2014). These bacterial strains are known also to
72 reductively debrominate bromoethenes (Ye, 2010), reported soil and groundwater contaminants
73 (Patterson et al., 2007). In this study, therefore, *S. multivorans* and *D. hafniense* PCE-S were
74 selected as model strains for a systematic comparison of stable isotope fractionation during
75 enzymatic debromination versus dechlorination.

76 Principally, the magnitude of isotope fractionation is determined by the rate-determining step of
77 a reaction. The carbon isotope effects during the expected rate-determining step for reductive
78 dehalogenation, the carbon-halogen bond cleavage, are expected to be similar based on the
79 comparison of theoretical maximum kinetic isotope effects (KIEs) with KIE_C =1.057 for C-Cl
80 (Elsner and 2005) and 1.043 for C-Br bond cleavage (Zakon, 2013). Bromine stable isotope
81 effects are, however, expected to be considerably smaller compared to chlorine stable isotope
82 effects considering KIE_{Br} = 1.002 (Zakon, 2013) compared to KIE_{Cl} = 1.013 (Elsner, 2010).
83 Previous reports for the reductive dehalogenation of chloroethenes, however, have already
84 shown that fractionation patterns are difficult to predict based on theoretical KIE alone but may
85 be masked by uptake and binding of the substrate to the enzyme influenced by the substrate

hydrophobicity and cell composition (Nijenhuis, 2005; Cichocka, 2007; Renpenning, 2015). The reaction mechanism of carbon-chlorine bond cleavage was reflected in dual-element, carbon-chlorine, fractionation patterns for trichloroethene (TCE) however, not for tetrachloroethene (PCE) during microbial dehalogenation (Cretnik, 2013; Cretnik, 2014; Renpenning, 2014). This variability was proposed to be associated with the relatively fast intrinsic reaction rate for PCE compared to its relatively slow overall transport rate. Therefore, stable isotope patterns are assumed to reflect reactions steps, such as enzyme binding, prior to bond cleavage (Renpenning, 2014). Similar effects were observed comparing the abiotic vs. biotic dihaloelimination of ethylene dibromide (Kuntze et al., 2016). Recently, in a computational modeling study, Ji et al. (2017) inferred that highly chlorinated ethenes (e.g. TCE and PCE) primarily react via an inner sphere nucleophilic substitution mechanism, whereas the less chlorinated ethenes (e.g. cis- and trans-DCE) mainly react through an inner sphere nucleophilic addition pathway. Although the exact mechanism of dehalogenation by cobamide-based dehalogenases is still under debate, several pathways can be considered: Co-C bond formation after direct Co(I) attack on the carbon backbone of the organohalide (Schrauzer et al., 1969); Co-X bond formation after direct Co(I) attack on the halogen atom (Payne et al., 2015); long-range electron transfer leading to substrate radical formation (Kunze et al., 2017).

Thus far, it is not clear if there are similarities or differences in the stable isotope fractionation for brominated and chlorinated analogues and if the fractionation patterns for brominated compounds can be predicted from their chlorinated analogues. In this study, therefore, the carbon and bromine stable isotope fractionation was investigated during reductive dehalogenation of bromoethenes by crude extracts of *S. multivorans* and *D. hafniense* PCE-S, for a direct comparison to previous results for chlorinated ethenes. Enrichment factors, ε_C and ε_{Br},

dual-element plots Λ (ε_C /ε_{Br}) and apparent kinetic isotope effects (AKIE) were determined for tribromoethene (TBE) and *cis/trans*-1,2-dibromoethene (DBE) and evaluated against those reported for their chlorinated analogs.

2. MATERIALS AND METHODS

2.1 Chemicals.

All chemicals were purchased from Sigma-Aldrich Chemie (Seelze, Germany) or Merck (Darmstadt, Germany) and were of analytical grade. Additionally, ethene was purchased from Airproducts (Hattingen, Germany), tribromoethene (TBE) and 1,2-dibromoethene (1,2-DBE; *cis*- and *trans*- mixture) were purchased from ABCR (Karlsruhe, Germany), and vinyl bromide (VB) was purchased from Aldrich.

2.2 Cultivation of bacteria and preparation of crude extracts.

S. multivorans (Miller et al., 1998) and *D. hafniense* strain PCE-S (Miller et al., 1997) were cultivated as previously described. During cultivation, PCE was provided as terminal electron acceptor and pyruvate as electron donor. Crude extracts were prepared in triplicate as previously described (Nijenhuis, 2005) under anoxic conditions (N_2/H_2 atmosphere) within an anoxic glovebox (Coy Laboratory Products Inc., USA), or under a steady stream of nitrogen gas.

2.3 Reductive debromination assays.

For determination of carbon and bromine stable isotope fractionation, reductive debromination assays were developed as previously described for reductive dechlorination (Nijenhuis, 2005). Provided as sole electron acceptor, either TBE or 1,2-DBE dissolved in ethanol were transferred at a final concentration of 1 mM to 4 ml reduced 1.6 mM methyl viologen buffer. Degradation was then facilitated by the addition of crude extracts of active enzyme and the extent of degradation was controlled by the addition of different concentrations of artificial electron acceptor titanium (III) citrate. Experiments were prepared in triplicate from three independent crude extracts. Abiotic controls were prepared for each assay to control for chemical reduction of the substrate by titanium(III) citrate and methyl viologen. All reactions were stopped by the addition of 1 mL saturated Na_2SO_4 (pH 1). Immediately following termination of reactions, 0.5 mL headspace was analyzed for concentrations of parent compound and products. Assays were kept at 4° C until stable isotope analysis.

2.4 Analytical methods.

A gas chromatograph (Chrompack CP-3800; Varian, Middelburg, The Netherlands) equipped with a flame ionization detector (GC-FID) and a GS-Q fused-silica capillary column (30 m x 0.53 mm; J&W Scientific, Waldbronn, Germany) was used for concentration analysis of samples. Analysis was performed as previously reported (Nijenhuis, 2005), and is described in detail in the Supplementary Information (SI).

Gas chromatography combustion isotope ratio mass spectroscopy (GC-C-IRMS) was used for carbon stable isotope analysis of samples. Analysis was performed as previously reported (Nijenhuis, 2005), and is described in detail in the SI.

149 Gas chromatography with multi-collector inductively coupled plasma mass spectrometry (GC-
150 MC-ICPMS) was used for bromine stable isotope analysis of samples. Analysis was performed
151 as previously reported (Gelman, 2010), and is described in detail in the SI.

152 ***2.5 Calculations and evaluation of data.***

153 Carbon and bromine isotope enrichment factors ($\varepsilon_{C \text{ or } Br}$) were determined for TBE and *cis*- and
154 *trans*-1,2-DBE according to the Rayleigh equation (Elsner and 2005):

155 $$\varepsilon \times ln\left(\frac{C_t}{C_0}\right) = ln\left(\frac{R_t}{R_0}\right) \quad [1]$$

156 where C_t and C_0 are the concentrations of the parent compound at time t and time 0 respectively,
157 and R = 1+ δ. $\varepsilon_{C \text{ or } Br}$ is reported in parts per thousand (‰). As assays were performed in
158 triplicate, data was combined in linear regressions (not forced through zero) used to generate
159 final ε values within a 95% confidence interval (95% CI) using Excel Analysis Toolpak
160 (Microsoft, USA).

161 The apparent kinetic isotope effect (AKIE) for carbon and bromine for each debromination
162 reaction was estimated using the following equation (Elsner, 2010):

163 $$AKIE = \frac{1}{(1 + z \times \varepsilon_{rp})} \quad [2]$$

164 where z is the number of atoms in intramolecular competition, and ε_{rp} is the reactive position-
165 specific ε which was calculated as follows:

166 $$\varepsilon_{rp} = \varepsilon \times \frac{n}{x}$$

167 [3]

where $\varepsilon_{C\ or\ Br}$ is the bulk enrichment, n is the number of atoms of the element considered in the molecule, and x is the number of atoms of the element considered at the reactive site.

Assuming reactions are stepwise and secondary isotope effects negligible, x = z, and after elimination upon combining equations 2 and 3, the term can be simplified as:

$$AKIE = \frac{1}{1+n\times \varepsilon} \quad [4]$$

C-Br dual-element fractionation correlation was expressed as Λ and determined by linear regression (not forced through zero) of δ^{81}Br data as a function of δ^{13}C data. As before, linear regressions were performed within a 95% CI using Excel Analysis Toolpak.

3. RESULTS

3.1 Dehalogenation and isotope fractionation of brominated ethenes

Reductive dehalogenation assays were prepared with crude extracts from *S. multivorans* and *D. hafniense* PCE-S. Figure 1. illustrates the pathway reported by Ye *et al.* (2010) and subsequently observed in this study. Dehalogenation occurred in all assays with TBE sequentially converted to VB via branched intermediates *trans-, cis-,* and 1,1-DBE, with *D. hafniense* PCE-S additionally producing small amounts of ethene.

3.2 Carbon stable isotope fractionation

TBE and 1,2-DBE carbon stable isotope fractionation was evaluated for crude extracts of both *S. multivorans* and *D. hafniense* strain PCE-S (SI Figure S1 & S3; Table 1). For *D. hafniense* PCE-S, ε_C was calculated at -7.7 ± 1.5‰, -9.5 ± 1.2‰ and -14.5 ± 0.7‰ for TBE, *trans*-1,2-DBE and

188 *cis*-1,2-DBE respectively. Fractionation of TBE was comparable to that reported for
189 dechlorination of TCE by *D. hafniense* PCE-S with ε_C =-10.9‰ (Cichocka, 2007), and is within
190 range of that reported for PCE at -5.2 to -8.9 (Nijenhuis, 2005; Cichocka, 2007).

191 For *S. multivorans*, however, a weak carbon isotope enrichment at ε_C = -1.2 ± 0.2‰ was
192 observed for TBE, while a one order of magnitude larger enrichment was measured at ε_C = -16.8
193 ± 1.8‰ and -21.2 ± 1.6‰ for *trans*- and *cis*-1,2-DBE, respectively. Observed carbon isotope
194 effects for TBE were much smaller than those reported for reductive dechlorination of TCE by *S.*
195 *multivorans*, where ε_C ranged from -13.2‰ to -26.0‰, however, were within range of that
196 reported for PCE dechlorination (ε_C = -0.4‰ to -2.2‰) (Nijenhuis, 2005; Lee, 2007; Cichocka,
197 2008; Renpenning, 2014; Renpenning, 2015).

198 $AKIE_C$ values were calculated and compared to theoretical KIE (Table 1). All values, ranging
199 from 1.035 ± 0.0036 to 1.044 ± 0.0032 for *S. multivorans* debrominating *trans*- and *cis*-1,2-DBE
200 respectively, and 1.016 ± 0.0030, 1.019 ± 0.0024, and 1.030 ± 0.0007 for *D. hafniense* PCE-S
201 debrominating TBE, *trans*-1,2,DBE and *cis*-1,2-DBE respectively, were similar (within the same
202 order of magnitude) to theoretical KIE calculated at 1.043 for a simple C-Br bond cleavage
203 (Zakon *et al.,* 2013). One notable exception was TBE debromination by *S. multivorans* which
204 was one order of magnitude smaller with an AKIE value of 1.002 ± 0.0004, a further indication
205 that in this particular reaction, either rate-limitation preceding C-Br bond cleavage contributes to
206 considerable masking effects or a different reaction mechanism is employed.

207 The occurrence of significantly smaller fractionation for *S. multivorans* debrominating TBE fits
208 with the proposition by Renpenning *et al* (2015) that substrate hydrophobicity may cause rate-
209 limitation at the active-site of the enzyme, resulting in masking of intrinsic isotope effects. It is

expected that this is an enzyme-specific effect, related to physico-chemical properties of the PceA reductive dehalogenase of *S. multivorans.* With the relative higher hydrophobicity of TBE (log K_{OW} = 3.20, (Canton and Wegman, 1983)), similar to that of PCE (log K_{OW} = 3.40, (Hansch et al., 1995)), compared to DBE (log K_{OW} = 1.76, (Hansch et al., 1995)) and TCE (log K_{OW} = 2.42, (Hansch et al., 1995)), this explanation is consistent with our results.

3.3 Bromine stable isotope fractionation

Similarly, bromine stable isotope fractionation was assessed (Table 1; SI Figure S2 & S4). ε_{Br} was estimated at -0.53 ± 0.15‰, -1.03 ± 0.26‰, and -1.18 ± 0.13‰ for *S. multivorans* debrominating *trans*-1,2-DBE, *cis*-1,2-DBE and TBE, respectively, and -0.97 ± 0.28, -1.16 ± 0.36, and -1.34 ± 0.32 for *D. hafniense* PCE-S debrominating *cis*-1,2-DBE, TBE and *trans*-1,2-DBE, respectively. Notably, no or little variability between microorganisms or within the debromination pathway was seen.

Generally, variability in bromine stable isotope fractionation between all assays was negligible. After correcting for non-reacting bromines within the molecule, all values were similar to the estimated Streitweiser limit at 1.002 (Table 1) with $AKIE_{Br}$ = 1.001 ± 0.0003, 1.002 ± 0.0005, and 1.004 ± 0.0004 for *S. multivorans* debrominating *trans*-1,2-DBE, *cis*-1,2-DBE and TBE respectively, and 1.002 ± 0.0006, 1.003 ± 0.0006, and 1.003 ± 0.0011 for *D. hafniense* PCE-S debrominating *cis*-1,2-DBE, *trans*-1,2-DBE and TBE respectively, nor was there any indication of a strong masking effect for bromine during debromination of TBE by *S. multivorans* as there was for carbon in the same assay. $AKIE_{Br}$ for this reaction did in fact exceed the estimated Streitweiser limit, as did $AKIE_{Br}$ for two other assays, but as all are approximations and are within the same order of magnitude, the difference is negligible.

3.4 Dual-element C and Br isotope analysis

Carbon stable isotope fractionation was plotted as a function of bromine stable isotope fractionation (Figure 1, Table 1), where linear regression slopes (expressed as $\Lambda \approx \varepsilon_C/\varepsilon_{Br}$) represent the combination of kinetic isotope effects for each assay. Dual-element slopes were estimated at 7.1 ± 1.6, 8.3 ± 3.7, and 8.9 ± 2.4 for *D. hafniense* PCE-S during debromination of *trans*-1,2-DBE, TBE and *cis*-1,2-DBE respectively (Table 1). Distinctly different and more variable values were estimated at 1.03 ± 0.2, 17.9 ± 5.8, and 29.9 ± 10.95 for *S. multivorans* during debromination of TBE, *cis*-1,2-DBE and *trans*-1,2-DBE respectively (Table 1). Again, this difference observed for TBE was similar for that for PCE, while DBE, behaved similar to TCE. This variability in isotope fractionation pattern for TBE may be due to slight differences in reaction mechanism, but, may also be due to different kinetics of the overall reaction, i.e. not C-Br bond cleavage but another, preceding, step in the reaction determining the observed isotope effect (Renpenning, 2014). In *S. multivorans*, the dehalogenation reaction mediated by PceA was recently suggested to function via long-range electron transport, leading to carbon-halogen bond cleavage, however, in case of TBE may be via an undirected electron transfer mechanism (Kunze et al, 2017; Ye et al 2010).

Discussion

4.1 Application of CSIA for assessment of in situ degradation of brominated organic compounds

Single-element, carbon stable isotope fractionation was previously reported for ethylene dibromide (EDB) (Henderson et al., 2008) and bromine stable isotope fractionation for brominated phenols (Bernstein, 2013), both for microbial dehalogenation. Dual-element carbon-bromine stable isotope fractionation was reported for several abiotic and biotic transformation

reactions of EDB (Kuntze et al., 2016) and abiotic degradation of tribromoneopentyl alcohol (TBNPA) (Kozell, 2015). Similarly, in our case significant carbon and bromine fractionation was observed for all tested bromoethenes, supporting the utility of CSIA for evaluating biotransformation of BOCs. Due to the observed variability in enrichment factors, a complementary approach including the assessment of the degrading microorganisms *in situ* may be necessary to allow a quantification of biodegradation applying the Rayleigh concept (Meckenstock et al., 2004; Nijenhuis and Kuntze, 2016).

4.2 Evaluation of the debromination reaction using CSIA

The ε_C values determined in this study, for what is assumed to be a stepwise, sequential hydrogenolysis reaction via single-electron transfer ranged from -7.5 ± 1.4‰ to -16.5 ± 2.8‰, with larger fractionation (when excluding the small *S. multivorans* TBE outlier) than that reported for the debromination of EDB (Henderson et al., 2008; Kuntze et al. 2016), in which reported ε_C were at -5.6 ± 1‰ and -5.3 ± 0.5‰ for ethylene dibromide (EDB) for biodegradation in anaerobic microcosms (Henderson et al., 2008) and by *S. multivorans* (Kuntze et al., 2016), respectively. Similarly, assuming a step-wise reaction for EDB, AKIE values for *cis*- and *trans*-DBE were also higher compared to EDB dehalogenation by *S.multivorans* with $AKIE_C$ = 1.044, 1.035 and 1.0107 (Kuntze et al., 2016), respectively. Similar differences were observed in bromine isotope fractionation with $AKIE_{Br}$ = 1.002, 1.001 and 1.0046 for *cis*- and *trans*-DBE and EDB (Kuntze et al., 2016), respectively, supporting a difference in the reaction mechanism between hydrogenolysis and dihaloelimination. The ε_{Br} values determined ranged from -0.53 ± 0.15‰ to -1.34 ± 0.32‰ and generally exceeded the values reported for microbial reductive debromination of brominated phenols at −0.20 ± 0.06‰ to −0.76 ± 0.08‰ (Bernstein, 2013), likely due to the difference in involved microbial community and enzymes as well as substrate

properties. Thus far, the described microorganisms and their reductive dehalogenases were reported to be highly substrate specific, each dehalogenating a restricted set of organohalides (Richardson, 2013). Structural differences in the enzyme structure may, therefore, affect the reaction mechanism to a certain extent (Kunze, 2017).

4.3 Rate-limitation and masking of isotope effects

The extent of fractionation for debromination observed in the present study was within the order of magnitude of that theoretically predicted by the semi-classical Streitweiser limit equation for C-Br bond cleavage for all reactions with the exception of debromination of TBE by *S. multivorans*. Here, carbon isotope fractionation was significantly lower at ε_C = -1.3 ± 0.2‰ than for all other assays, including debromination of TBE by *D. hafniense* PCE-S at ε_C = -7.5 ± 1.4‰, and was remarkably similar to the previously reported small ε_C for *S. multivorans*-mediated reductive dehalogenation of PCE at -0.4‰ to -2.2‰ (Nijenhuis, 2005; Cichocka, 2007; Renpenning, 2015). This extent of masking was not seen for bromine during debromination of TBE by *S. multivorans*, with ε_{Br} evaluated at -1.18 ± 0.13‰, an average value within all assays which ranged from -0.53 ± 0.15 for *S. multivorans* debrominating *trans*-1,2-DBE to -1.34 ± 0.32‰ for *D. hafniense* PCE-S debrominating *trans*-1,2-DBE. As fractionation patterns were different for carbon than for bromine, the correlation between carbon and bromine fractionation was variable, with $\Lambda_{C\text{-}Br}$ ranging from 7.1 ± 1.6‰ to 8.9 ± 2.4‰ for *D. hafniense* PCE-S, and from 1.03 ± 0.2‰ to 29.9 ± 10.95‰ for *S. multivorans.* This variability between strains and, most pronouncedly for *S. multivorans* within the dehalogenation pathway, suggests differences in the reaction between these substrates at a single enzyme, similarly to the previous observations for the chlorinated ethenes (Cretnik, 2013; Wiegert, 2013; Renpenning, 2014).

5. Implications for the application of CSIA for assessment of *in situ* biodegradation of BOC

BOCs have gained the interest of policy makers and environmental researchers alike due to their toxicity and suspected carcinogenic, endocrine disruptive, and neurodegenerative properties (de Wit, 2002; Alaee, 2003), and have been restricted or even banned by regulatory agencies such the U.S. Environmental Protection Agency (EPA) (Betts, 2008). However, due to their persistent nature, as evidenced by detection in house dust, soil, and water samples, as well as in human breast milk (de Wit, 2002), BOCs continue to pose a threat to human health and ecosystems, and their environmental fate and transport should be elucidated. The use of stable isotope analysis to detect and characterize transformation of BOCs *in situ* may be a valid option as fractionation has been reported for both biotic and abiotic processes. And while much work is still needed, this study now adds to the still quite small database of measurable and statistically significant carbon and bromine isotope enrichment factors determined for microbial reductive debromination.

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Tables and Figures

Table 1. Carbon and bromine isotope fractionation during enzymatic reductive debromination of TBE, and *cis*- and *trans*-1,2-DBE expressed as enrichment factors (ε_C and ε_{Br}) and AKIE values assuming reactions are stepwise and secondary isotope effects negligible. Sample size is given as N. 2-D analysis was also performed and linear regression slopes are denoted Λ.

	S. multivorans						*D.hafniense* PCE-S					
	$\varepsilon_{C\ [‰]}$		**R^2**	**N**	**$AKIE_C$**		**$\varepsilon_{C\ [‰]}$**		**R^2**	**N**	**$AKIE_C$**	
TBE	-1.2	±0.2	0.829	39	1.002	±0.0004	-7.7	±1.5	0.799	30	1.016	±0.0030
cis-DBE	-21.2	±1.6	0.937	48	1.044	±0.0032	-14.5	±0.7	0.988	23	1.030	±0.0007
trans-DBE	-16.8	±1.8	0.939	25	1.035	±0.0036	-9.5	±1.2	0.870	42	1.019	±0.0024
	$\varepsilon_{Br\ [‰]}$				**$AKIE_{Br}$**		**$\varepsilon_{Br\ [‰]}$**				**$AKIE_{Br}$**	
TBE	-1.18	±0.13	0.937	27	1.004	±0.0004	-1.16	±0.36	0.828	13	1.003	±0.0011
cis-DBE	-1.03	±0.26	0.912	10	1.002	±0.0005	-0.97	±0.28	0.777	18	1.002	±0.0006
trans-DBE	-0.53	±0.15	0.911	9	1.001	±0.0003	-1.34	±0.32	0.876	14	1.003	±0.0006
	Λ						**Λ**					
TBE	1.0	±0.2	0.814	25			8.3	±3.7	0.711	12		
cis-DBE	17.9	±5.8	0.862	10			8.9	±2.4	0.797	17		
trans-DBE	29.9	±11.0	0.856	9			7.1	±1.6	0.885	14		

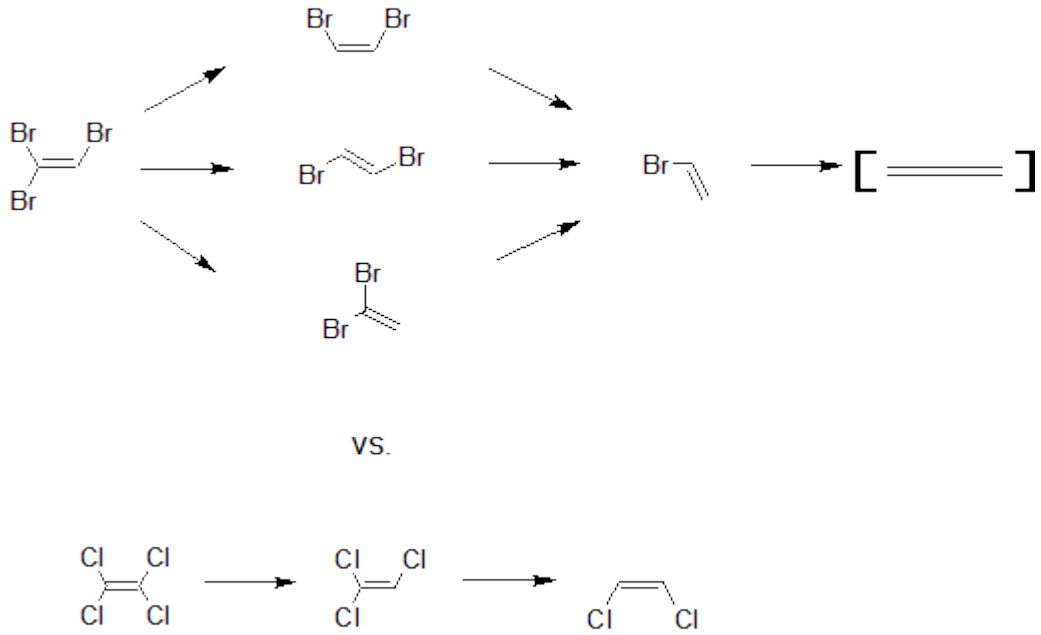

Figure 1. Enzymatic reductive debromination versus reductive dechlorination catalyzed by crude extracts of *Sulfurospirillum multivorans* and *Desulfitobacterium hafniense* PCE-S. Tribromoethene is sequentially debrominated to vinyl bromide via branched intermediates 1,2-*cis*- and *trans*-, and 1,1-dibromoethene, compared to the exclusive formation of *cis*-1,2-DCE as final product of dechlorination. Additionally, small amounts of ethene are produced when the debromination reaction is catalyzed by crude extracts of *D. hafniense* PCE-S.

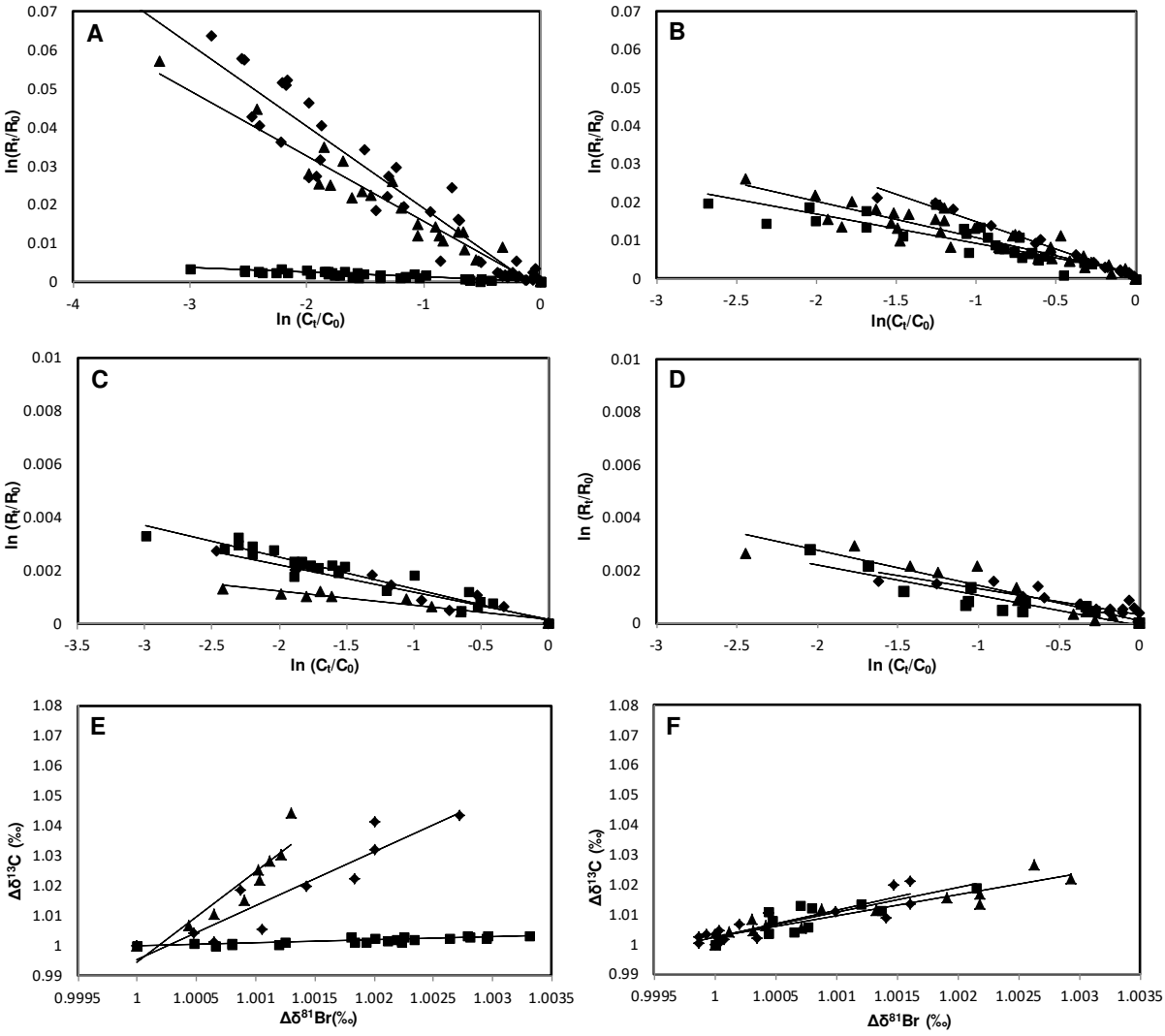

Figure 2: Rayleigh plots of carbon (A,B) and bromine (C,D) stable isotope fractionation and dual-element plots (E,F) for TBE (square), *cis*-DBE (diamant) and *trans*-DBE (triangle) dehalogenation by *S. multivorans* (A,C,E) and *D. hafniense* strain PCE-S (B,D,F).