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

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HIGHLIGHTS

- Microbial reductive debromination of tribromoethene and dibromoethene.
- Significant carbon and bromine stable isotope fractionation during debromination.
- Variable dual-element slopes were observed suggesting variability in the reaction.
- CSIA can be used to assess the fate of brominated compounds *in situ*.

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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* ($\varepsilon_C = -1.3 \pm 0.2$‰) compared to *D. hafniense* ($\varepsilon_C = -7.7 \pm 1.5$‰). However, higher fractionation was observed for dibromoethene (DBE) dehalogenation by *S. multivorans* ($\varepsilon_C = -16.8 \pm 1.8$‰ and -21.2 ± 1.6‰ for *trans*- and *cis*-1,2- (DBE) respectively), compared to *D. hafniense* PCE-S ($\varepsilon_C = -9.5 \pm 1.2$‰ 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* ($\varepsilon_{Br} = -0.53 \pm 0.15$‰, -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 ($\varepsilon_{Br} = -0.97 \pm 0.28$‰, -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 ($\varepsilon_C/\varepsilon_{Br}$) = 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.

1. Introduction

Several chlorinated as well as brominated organic compounds (BOCs) are pervasive environmental contaminants (de Wit, 2002; Alaee et al., 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-

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

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

Principally, the magnitude of isotope fractionation is determined by the rate-determining step of a reaction. The carbon isotope effects during the expected rate-determining step for reductive dehalogenation, the carbon-halogen bond cleavage, are expected to be similar based on the comparison of theoretical maximum kinetic isotope effects (KIEs) with $KIE_C = 1.057$ for C–Cl (Elsner et al., 2005) and 1.043 for C–Br bond cleavage (Zakon et al., 2013). Bromine stable isotope effects are, however, expected to be considerably smaller compared to chlorine stable isotope effects considering $KIE_{Br} = 1.002$ (Zakon et al., 2013) compared to $KIE_{Cl} = 1.013$ (Elsner et al., 2010). Previous reports for the reductive dehalogenation of chloroethenes, however, have already shown that fractionation patterns are difficult to predict based on theoretical KIE alone but may be masked by uptake and binding of the substrate to the enzyme influenced by the substrate hydrophobicity and cell composition (Nijenhuis et al., 2005; Cichocka et al., 2007; Renpenning et al., 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 et al., 2013, 2014; Renpenning et al., 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 et al., 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 and Deutsch, 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 ($\varepsilon_C/\varepsilon_{Br}$) 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 et al., 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 et al., 2005). Provided as sole electron acceptor, either TBE or 1,2-DBE dissolved in ethanol were transferred at a final concentration of 1 mM–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