Research Brief 155: Assessing Bioremediation of Chloroethenes through Stable Carbon Isotope Fractionation

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Background: Chloroethenes, such as tetrachloroethene (PCE), trichloroethene (TCE), isomers of dichloroethene (DCE), and vinyl chloride (VC), are among the most prevalent and problematic groundwater pollutants. These compounds pose serious health threats due to their toxicity and potential carcinogenicity. *In situ* bioremediation is often used at contaminated sites, but it is difficult to quantify the effectiveness of the biological processes because of changes that can result from physical processes, such as dissolution, volatilization, and sorption.

Dr. Lisa Alvarez-Cohen at the University of California, Berkeley SBRP believes that quantification of shifts in stable carbon isotope ratios is a promising approach for evaluating the effectiveness of in situ bioremediation systems. Physical processes cause minor or negligible shifts in isotope ratios, while biological conversion of organic compounds can cause significant changes in the ratio of carbon isotopes (^{13}C : ^{12}C) in both the reactants and products. In biological transformations, reaction rates tend to be faster for molecules with light isotopes than for molecules with heavy isotopes. As a result, the residual reactant becomes enriched with the heavy isotopes and the daughter products are enriched with the light isotopes. This enrichment (fractionation) can be measured using compound-specific stable isotope analysis.

In situ bioremediation of TCE can occur by microbial reductive dehalogenation. The process involves the sequential reduction of TCE to DCE, followed by conversion of DCE to VC (a known human carcinogen), and finally VC to the innocuous end product ethene.

Advances: Early fractionation studies performed with mixed bacterial cultures from field samples yielded highly variable isotopic fractionation effects. This suggests that different organisms utilizing different pathways to degrade chloroethene compounds may cause different isotopic shifts.

Dr. Alvarez-Cohen's research group studied stable carbon isotope fractionation during the reduction of TCE using three test systems representing phylogenetically distinct organisms:

- Pure cultures of bacterial strains that are known to dechlorinate chloroethenes completely to ethene (*Dehalococcoides ethenogene*s 195 and *Dehalococcoides* sp. strain BAV1)
- A well-characterized mixed culture containing *Dehalococcoides*
- Pure isolates of bacterial strains that dechlorinate TCE to DCE, but do not complete the metabolism through VC to ethene (*Sulfurospirillum multivorans* and *Dehalobacter restrictus* strain PER-K23)

This is the first measurement of carbon isotope fractionation by *Dehalococcoides* isolates and of TCE transformation by *S. multivorans* and *D. restrictus* strain PER-K23. The extent of fractionation varied widely, yet within each culture constant enrichment factors were observed. Dechlorinating cultures exhibited a range of enrichment factors at each dechlorination step, and strains within the same genus or species generated significantly different enrichment factors. Additionally, isotope fractionation generated by a microbial community was quite different from that generated by isolates. These results offer an interesting comparison and insight into factors that affect biological fractionation.

The large range of TCE enrichment factors observed, coupled with the reported similarities of the reductive dehalogenase enzymes (RDases), suggest that these biological fractionations are governed by a combination of the structure of the native enzyme and its cofactor, transport, and enzyme-

substrate binding prior to the carbon-chlorine bond-breaking step. As a result of these findings, Dr. Alvarez-Cohen believes that general categorization of enrichment factors without specific measurements can result in misinterpretation and that caution should be exercised in selecting appropriate values for quantitative analysis to predict the extent of dechlorination.

Significance: Compound-specific stable isotope analysis is a valuable tool for assessing *in situ* bioremediation at contaminated sites. However, as a result of wide variation in enrichment factors measured for both bacterial isolates and enriched communities, this study found that it is important to estimate site representative factors to optimize estimates of degradation rates.

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To learn more about this research, please refer to the following sources:

Lee, Patrick K H, Mark E. Conrad, and Lisa Alvarez-Cohen. 2007. Stable carbon isotope fractionation of chloroethenes by dehalorespiring isolates. Environmental Science and Technology (http://pubs.acs.org/journals/esthag/). 41(12):4277-85.