



Assessment of chloroethene biodegradation in the subsurface by microbiological, molecular and isotopic tools

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A multiple lines of evidence approach to assess the biodegradation potential of contaminated sites includes

- site investigation analysing pollutant distribution (compounds, concentrations, isotopic composition) and hydrochemical conditions (redox conditions)

- determination of the presence of pollutant degrading bacteria in the field by microbiological (most probable number, MPN) and molecular (polymerase chain reaction, PCR) methods

- analysis of degradation processes in the laboratory by microcosms with determination of site specific isotopic enrichment factors enabling the quantification of biodegradation processes in the field.

Results will be shown of the application of such a multiple lines of evidence approach at a chloroethene-contaminated site in Frankenthal, Germany.

In anaerobic groundwater microcosms, reductive transformation of perchloroethene (PCE) and trichloroethene (TCE) was observed to mainly proceed to cis-1,2-dichloroethene (cDCE). 16S-PCR analysis showed a wide distribution of halo-respiring bacteria capable of PCE degradation to cDCE, whereas *Dehalococcoides* – the only organisms described so far being able of complete reductive dechlorination down to ethene – was only found in one groundwater sample. Aerobic microcosms showed metabolic degradation of the lower chlorinated compounds cDCE and vinyl chloride (VC). Co-metabolic degradation of cDCE with VC as auxiliary substrate occurred, too. Significant stable carbon isotope fractionation was observed during anaerobic degradation of PCE and TCE as well as during aerobic degradation of cDCE and VC.

Compiling the results of the different assessment methods, sequential dechlorination – PCE/TCE to cDCE anaerobically and cDCE to CO₂ aerobically – was demonstrated to occur at the Frankenthal site. The extent of biodegradation in the field was calculated based on the enrichment factors determined in microcosms and the ¹³C-isotopic composition of the contaminants on site.

The application of molecular methods is continuously increasing. For example, microbiological and molecular tools showed the presence and activity of halo-respiring bacteria in sediment samples of the Yangtze river, China. PCR-detection demonstrated the presence of five different halo-respiring bacterial groups as well as of four different dechlorinating enzymes of *Dehalococcoides*.

In conclusion, our study demonstrates that (i) multiple lines of evidence approaches result in a profound understanding of the biodegradation processes occurring in the field, (ii) stable isotope fractionation is suitable for assessing and quantifying anaerobic and aerobic chloroethene degradation and (iii) detection and quantification of dechlorinating bacteria and enzymes by PCR methods provide more insight into biodegradation processes.

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