# Assessment of *in situ* degradation of chlorinated ethenes and bacterial community structure in a complex contaminated groundwater system

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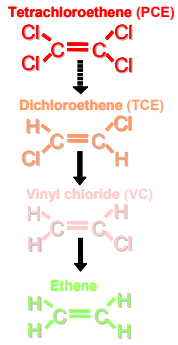
## Introduction

**The occurrence of *in situ* degradation of chlorinated ethenes was investigated in several geological units of a complex groundwater system in Bitterfeld, Germany. The hydrogeochemistry and the distribution of chlorinated ethenes was assessed using multivariate analysis. The *in situ* biodegradation was evaluated by stable isotope analysis, and biomarkers (16S rRNA) were applied to detect specific dehalorespiring genera. In parallel, the changes of the microbial community composition in the aquifers were evaluated using variation partitioning analysis. The concentration and isotope fractionation analysis revealed that chlorinated ethenes were subjected to substantial biodegradation. Taxon-specific investigation indicated the simultaneous presence of various potential dehalorespiring populations (*Dehalococcoides*, *Desulfuromonas*, *Dehalobacter*) in several wells. Variation partitioning analysis of the bacterial community structure in the contaminated groundwater system indicated a predominant effect of the chlorinated ethenes concentrations (56.3% of the variance, *P*=0.005).**

## Biodegradation and reductive dechlorination


Chlorinated ethenes can be dechlorinated to the harmless product of ethene under anaerobic condition

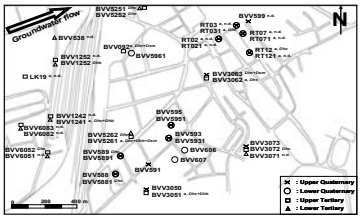
## Experimental set-up


Groundwater sampling

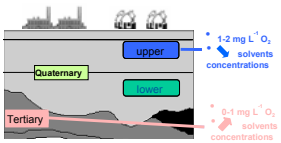



[1]R: (2007). The R Foundation for Statistical Computing.
[2]Legendre&Legendre. Numerical Ecology (1998).

## Study area




•Bacterial community patterns available
*Dhc* = *Dehalococcoides* sp., *Dhb* = *Dehalobacter* sp.,
*Dsm* = *Desulfuromonas* sp., n.d. = not detected.




## Chemical and hydrogeological charaterization

### 1) Quaternary aquifer

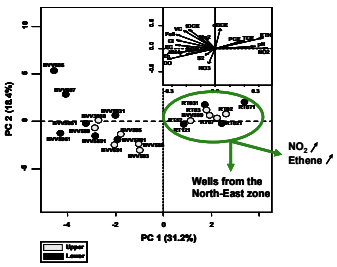
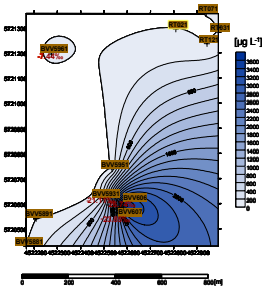



**Fig.** Ordination plot generated by principal component analysis representing the relationship between the wells, the contaminant concentrations and geochemical variable.

### 2) Isotope fractionation of vinyl chloride in the lower quaternay aquifer[3]



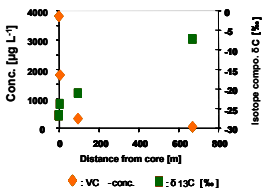
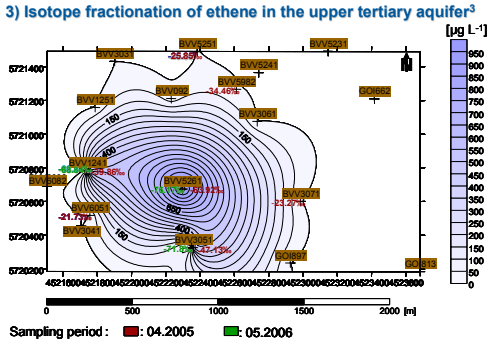




**Fig.** Decrease in VC concentrations associated with a significant enrichment in $\delta^{13}C$ along the flow path: an evidence for *in situ* biodegradation.

Sampling period: 12.2005
Analytical error associated with the isotope analysis: ± 0.5 ‰

### 3) Isotope fractionation of ethene in the upper tertiary aquifer[3]




[3] Mapping: Surfer Version 7. 02. Surface Mapping System, Golden Software, Inc.

## Molecular analysis

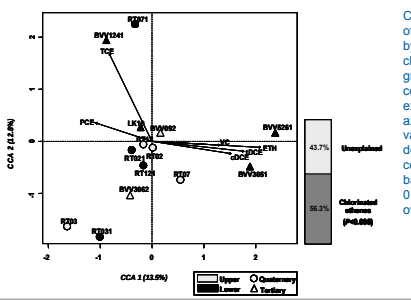
### 1) Taxon specific 16S rRNA-based PCR amplification to detect dehalogenators

| | Number of wells | *Dehalococc.* sp. | *Desulfurom.* sp. | *Dehalobact.* sp. |
|---|---|---|---|---|
| Upper Quaternary | 5 | 2 | 0 | 0 |
| Lower Quaternary | 4 | 3 | 0 | 1 |
| Upper Tertiary | 8 | 5 | 2 | 3 |
| Lower Tertiary | 9 | 3 | 0 | 2 |

Taxon-specific 16S rRNA-based PCR amplification was used to test the presence of three potential dehalogenators in the groundwater samples. Positive signals were yielded in 15 of the 27 selected groundwater samples.

- Potential complex dehalogenating bacterial community: several taxa per well
- Systematic detection of *Dehalococcoides sp.* in all geological layers

### 2) Influence of contaminant concentrations on the bacterial community structure




Canonical correspondence analysis (CCA) of bacterial community patterns generated by 16S rRNA DGGE analysis for different chlorinated ethenes contaminated groundwater samples. Chlorinated ethenes concentrations are used as quantitative explanatory variables. The value % on the axes indicates the percentage of total variation which they explain. The variance decomposition of the CCA on the bacterial community patterns is shown as a diagram bar. Sum of all canonical eigenvalues, 0.922; total inertia, 1.636; Monte Carlo overall permutation test, *P*=0.005.

## Conclusions

- **Principal component analysis can be employed for analysing spatially and temporally the major trends of variation of the geochemistry and the contaminant patterns of geologically complex contaminated groundwater systems**
- **Evidence for natural attenuation of PCE and TCE to ethene was provided in several geological units of the groundwater system using multiple distinct but converging lines of evidence**
- **Isotope signatures were used as a spatial and temporal indicator for biodegradation: in the initial phase, the product is lighter than the educt, and as the biodegradation proceeds, the product become heavier**
- **Molecular investigations showed a complex bacterial community potentially associated with *in situ* biodegradation of chlorinated ethenes in several geological units**
- **Variation partitioning analysis of the bacterial community structure in the contaminated groundwater system indicated a predominant effect of the chlorinated ethenes concentrations (56.3% of the variance, *P*=0.005)**

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