

## **Integrated plume characterisation including isotopic fractionation and molecular biological tools documents degradation of DCE and VC under iron reducing conditions in a deep aquifer**

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**Abstract** Recent developments in compound specific carbon and chlorine stable isotope analysis and in molecular biological tools for quantification of specific degraders have enabled the use of these novel tools for evaluation of natural degradation in solvent plumes. A PCE contamination at a former dry cleaning facility in Rødékro, Denmark, has resulted in a 2 km long and 70 m deep plume of PCE and its metabolites in the groundwater aquifer. The original field data indicated degradation of PCE by reductive dechlorination to TCE and DCE. However, further degradation of DCE in the plume was questionable, and the DCE degradation pathway was unknown, as VC concentrations are very low. Adequate assessment of the risk for the drinking water resource required better evaluations of the degradation of DCE and VC in the plume. A combined approach including analysis for chlorine and carbon isotopic fractionation and specific degraders (*Dehalococcoides* Sp. cells Dhc and vinylchloride reductase genes *vcrA*) as well as traditional investigations was applied. Selected multilevel wells centrally placed along the length of the plume were sampled for the combined approach analysis. PCE and TCE transformation under manganese to iron reducing conditions was documented by <sup>13</sup>C enrichment in PCE and TCE and significant production of cis-DCE. Cis-DCE and VC degradation under iron reducing conditions was documented by <sup>13</sup>C enrichment in cis-DCE and strong <sup>13</sup>C enrichment in VC in the far end of the plume. Comparison of isotopic fractionation for chlorine and carbon indicated that reductive dechlorination is the main transformation process for cis-DCE. In addition, the presence of Dhc was indicated by molecular analysis in wells with high <sup>13</sup>C enrichment in cis-DCE. In the far end of the plume, where significant VC degradation occurs, the average carbon isotope ratio of all chlorinated ethenes started to deviate from the source isotope ratio. This may indicate that VC is degraded by other processes than reductive dechlorination, which is consistent with the absence of ethene.

**Key words** isotopic fractionation; chlorinated solvents; natural degradation; specific degraders; degradation pathway

## INTRODUCTION

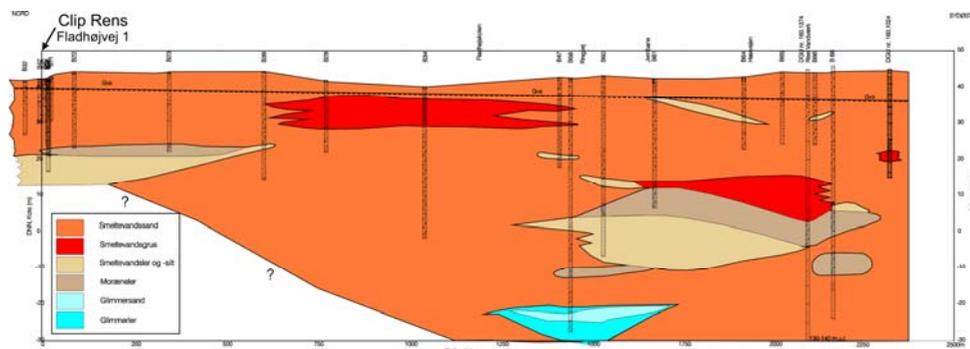
A perchloroethylene (PCE) contamination at a former dry cleaning facility in Rødekro, Denmark, has resulted in a 2 km long and 70 m deep plume of PCE and its metabolites in the groundwater aquifer. The original field data indicated degradation of PCE by reductive dechlorination to trichloroethylene (TCE) and dichloroethylene (DCE). However, further degradation of DCE in the plume was questionable, and the DCE degradation pathway was unknown, as vinylchloride (VC) concentrations are very low. Adequate assessment of the risk for the drinking water resource required better evaluations of the degradation of DCE and VC in the plume.

An integrated plume characterization was applied involving analysis for traditional redox parameters, chlorinated ethenes and related metabolites, chlorine and carbon isotopic fractionation and specific degraders (*Dhc* and *vcrA*).

## SITE DESCRIPTION

The site is the former location for a large dry-cleaning facility. The activities of the former dry-cleaning facility has lead to severe soil and groundwater contamination, including free phase DNAPL in the aquifer, at the site /1/.

The site is located in an area just west of the last ice-age glacier front. The sediments predominantly consists of meltwater sands and gravel /1/. A geological profile is shown in Figure 1.

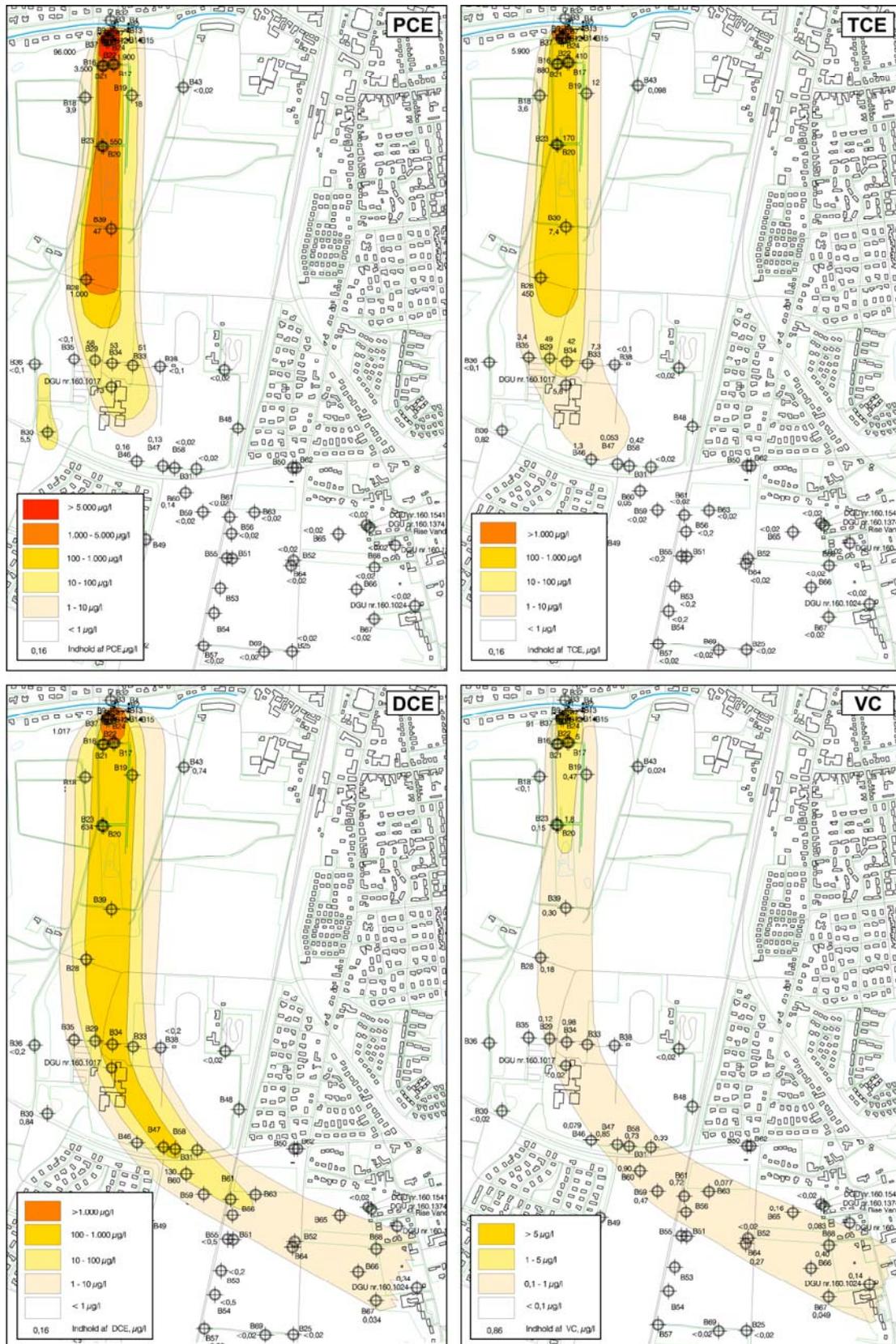


**Fig. 1** Geologic profile for the aquifer at the former dry cleaning facility /1/. Orange and red is sand and gravel. Beige and brown is silt and clay.

The contamination at the site has resulted in a 2 km long and 70 m deep plume of PCE and its metabolites in the sandy groundwater aquifer. The contaminant plume is illustrated for PCE, TCE, DCE and VC in Figure 2.

## DEGRADATION OF PCE

Under anaerobic conditions PCE can be degraded by reductive dechlorination via TCE and DCE to VC and finally ethene and ethane. Complete dechlorination of the metabolites DCE and VC requires strongly reducing conditions (iron to sulphate reducing or methanogenic) and the presence of specific degraders, *Dehalococcoides* Sp. (*Dhc*) and *Dhc* with vinylchloride reductase genes (*vcrA*).



**Fig. 2** The plume originating from the dry-cleaning facility illustrated for PCE and the metabolites TCE, DCE and VC /1/. Note different concentration range for each figure.

Another possible degradation pathway for DCE and VC under manganese to iron reducing conditions is anaerobic oxidation to CO<sub>2</sub>.

## INVESTIGATIONS

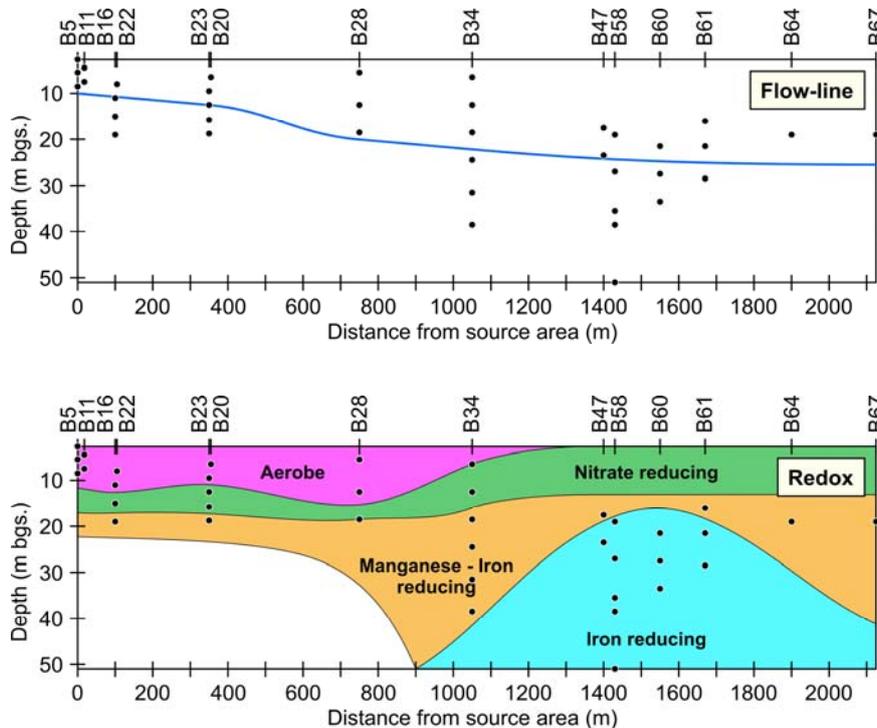
Selected multilevel wells centrally placed along the length of the plume were sampled for the combined approach analysis. Traditional investigations included analysis for PCE and its degradation products (TCE, *cis*-DCE, *trans*-DCE, 1,1-DCE, VC and ethene) and for redox sensitive parameters (oxygen, nitrate, dissolved manganese, dissolved iron, sulphate, sulphide and methane). PCE and degradation products were analyzed by GC-MS. An-ions were analysed by IC and cat-ions by AAS.

Isotopic fractionation analysis for carbon in PCE, TCE, *cis*-DCE and VC and chlorine in *cis*-DCE were conducted by GC coupled with isotope-ratio MS /2, 3/. Specific bacteria analyses were conducted by DNA extraction followed by quantitative PCR /4/.

## RESULTS

### Flow-line and redox conditions

An approximate flow-line along the length of the plume, following approximate maximum concentrations of the sum of chlorinated ethenes, is illustrated in Figure 3. An illustration of the redox conditions based on interpretation of the measured redox sensitive parameters is also illustrated in Figure 3.



**Fig. 3** Approximate flow-line along the length of the contaminant plume and redox conditions based on interpretation of measured redox sensitive parameters.

Conditions in the upper part of the aquifer are aerobic to nitrate reducing, whereas conditions in the deeper part of the aquifer are manganese to iron reducing and in parts more strongly iron reducing.

### PCE and metabolites

The molar concentrations of PCE and its metabolites are illustrated in Figure 4.

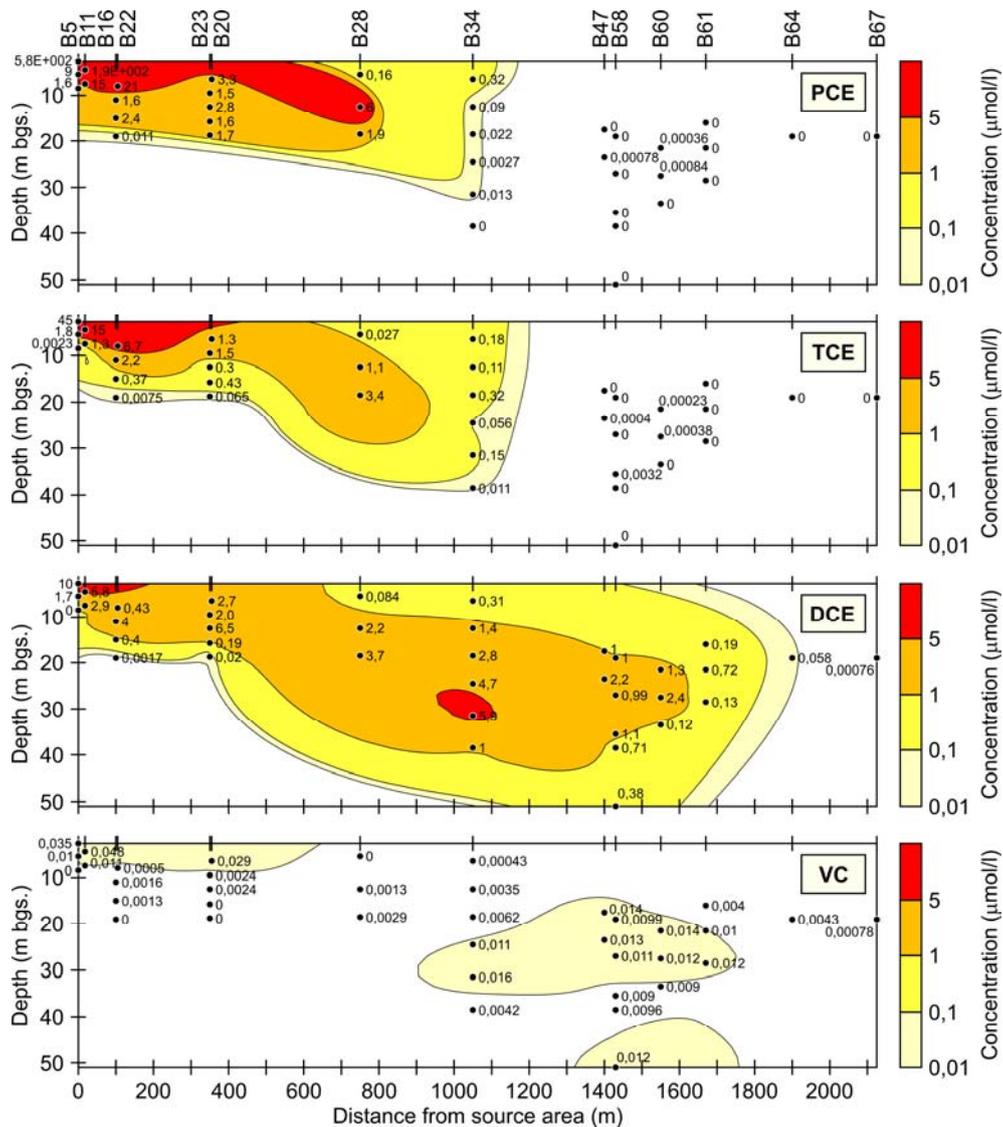


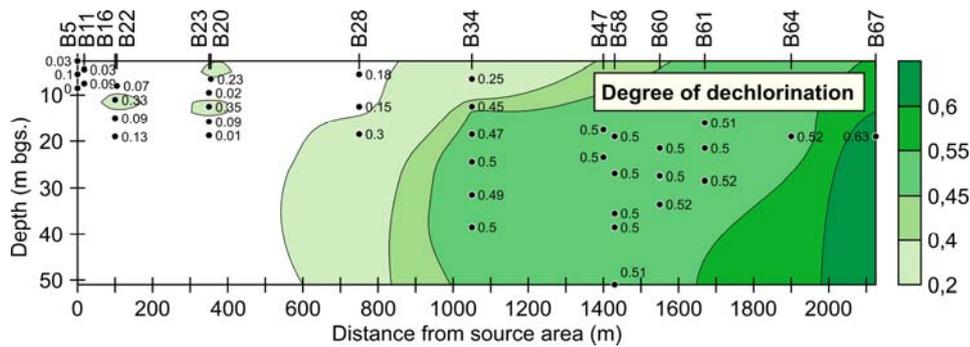
Fig. 4 Molar concentrations of PCE and its metabolites TCE, DCE and VC in the plume.

PCE and TCE transformation by reductive dechlorination under manganese to iron reducing conditions is documented by significant production of cis-DCE. Cis-DCE degradation by reductive dehalogenation to VC under iron reducing conditions is indicated by apparent VC production in the downstream end of the plume. However, VC concentrations are very low, and no production of ethylene and/or ethane is observed.

The degree to which a chlorinated compound has been degraded by reductive dehalogenation can be illustrated by the degree of dechlorination (D), expressed as:

$$D = \frac{c_{TCE} + 2 \cdot c_{DCE} + 3 \cdot c_{VC} + 4 \cdot (c_{ethene} + c_{ethane})}{4 \cdot (c_{PCE} + c_{TCE} + c_{DCE} + c_{VC} + c_{ethene} + c_{ethane})} \quad (1)$$

The degree of dechlorination in the plume is illustrated in Figure 5.



**Fig. 5** The degradation of PCE by reductive dechlorination illustrated by the degree of dechlorination of PCE in the plume.

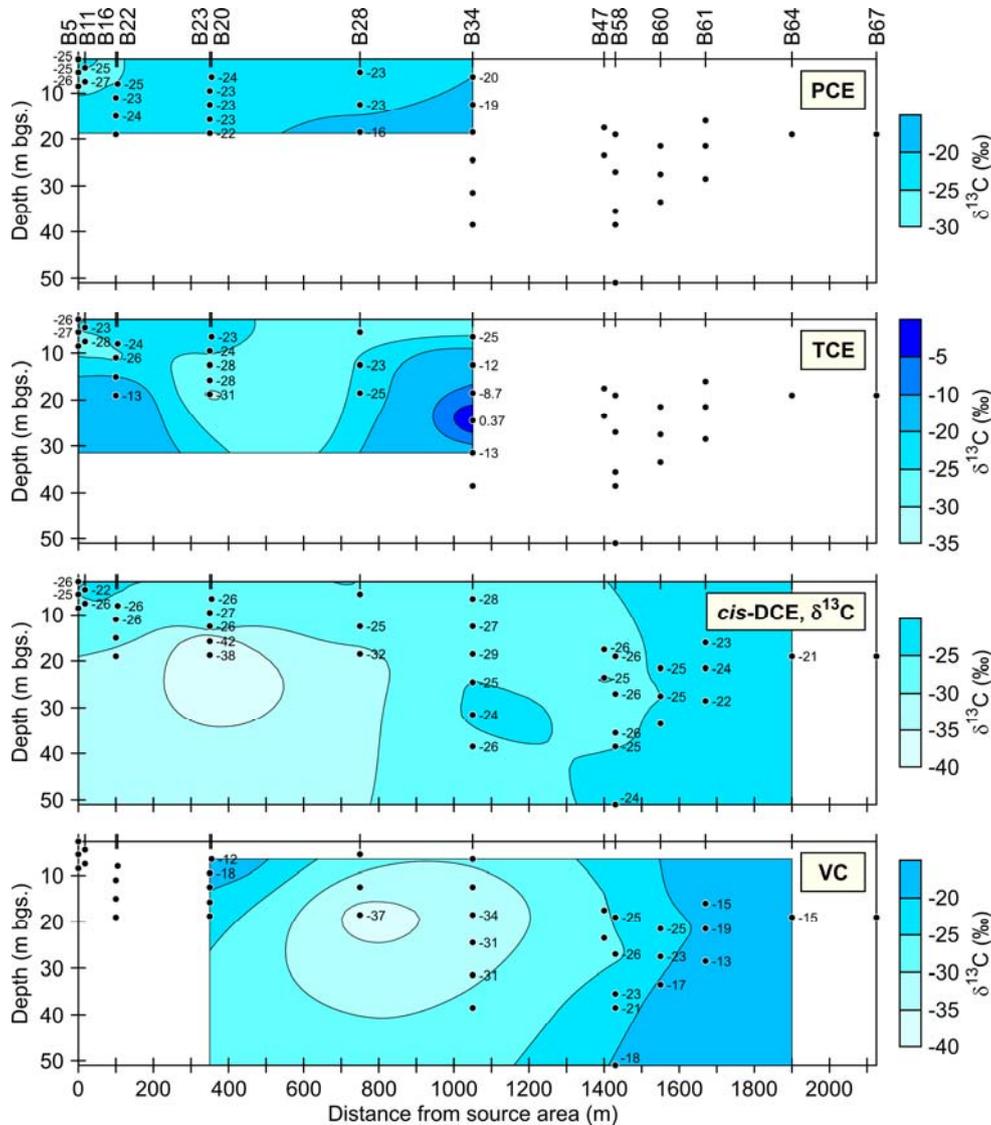
In the manganese-iron reducing and iron reducing zones the degree of dechlorination is about 0.5 for almost all the wells, confirming that PCE is completely degraded to cis-DCE and suggesting that further degradation of cis-DCE by reductive dechlorination to VC and ethane/ethane may be insignificant. In the well furthest downgradient, a higher degree of dechlorination is found. However, in a flow-system the value may be biased by the higher mobility of VC than cis-DCE.

### Isotopic fractionation

Carbon and chlorine in PCE and other chlorinated organic compounds consist of 2 stable isotopes  $^{12}\text{C}$  and  $^{13}\text{C}$  and  $^{35}\text{Cl}$  and  $^{37}\text{Cl}$ , respectively. When PCE and its degradation products are degraded by reductive dechlorination or oxidation, an increase is observed in the content of the heavy isotopes,  $^{13}\text{C}$  and  $^{37}\text{Cl}$ , respectively. This is referred to as isotopic fractionation. The fraction of  $^{13}\text{C}$  in the degradation product is initially low and then increase to the initial fraction for the mother compound.

An isotopic enrichment trend along a flow-line is evidence of biodegradation of the compound. Preliminary results for the isotopic fractionation of carbon in PCE, TCE, cis-DCE and VC given as  $\delta^{13}\text{C}$  notation are illustrated in Figure 6.

PCE and TCE transformation under manganese to iron reducing conditions is documented by  $^{13}\text{C}$  enrichment in PCE and TCE. Cis-DCE and VC degradation under iron reducing conditions is documented by  $^{13}\text{C}$  enrichment in cis-DCE and strong  $^{13}\text{C}$  enrichment in VC in the far end of the plume. Comparison of isotopic fractionation for chlorine (data not shown) and carbon indicate that reductive dechlorination is the main transformation process for cis-DCE.



**Fig. 6** Isotopic fractionation of carbon for PCE, TCE, cis-DCE and VC. Preliminary results.

### Specific degraders

Molecular analysis indicated the presence of Dhc (though below quantification level) in two wells with high  $^{13}\text{C}$  enrichment in cis-DCE. No *vcrA* genes were detected, but the detection limit was much higher than for Dhc detection.

### CONCLUSIONS

PCE and TCE transformation under manganese to iron reducing conditions was documented by  $^{13}\text{C}$  enrichment in PCE and TCE and significant production of cis-DCE. Cis-DCE and VC degradation under iron reducing conditions was documented by  $^{13}\text{C}$  enrichment in cis-DCE and strong  $^{13}\text{C}$  enrichment in VC in the far end of the plume. Comparison of isotopic fractionation for chlorine and carbon indicated that reductive dechlorination is the main transformation process for cis-DCE.

The presence of Dhc was indicated by molecular analysis in wells with high  $^{13}\text{C}$  enrichment in cis-DCE. In the far end of the plume, where significant VC degradation occurs, the average carbon isotope ratio of all chlorinated ethenes started to deviate from the source isotope ratio. This may indicate that VC is degraded by other processes than reductive dechlorination, which is consistent with the absence of ethene. These findings are of great importance for the risk assessment of the site.

## REFERENCES

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