

Assessing chlorinated ethene degradation in a large scale contaminant plume by dual carbon–chlorine isotope analysis and quantitative PCR

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A B S T R A C T

The fate of chlorinated ethenes in a large contaminant plume originating from a tetrachloroethene (PCE) source in a sandy aquifer in Denmark was investigated using novel methods including compound-specific carbon and chlorine isotope analysis and quantitative real-time polymerase chain reaction (qPCR) methods targeting *Dehalococcoides* sp. and *vcrA* genes. Redox conditions were characterized as well based on concentrations of dissolved redox sensitive compounds and sulfur isotopes in SO_4^{2-} .

In the first 400 m downgradient of the source, the plume was confined to the upper 20 m of the aquifer. Further downgradient it widened in vertical direction due to diverging groundwater flow reaching a depth of up to 50 m. As the plume dipped downward and moved away from the source, O_2 and NO_3^- decreased to below detection levels, while dissolved Fe^{2+} and SO_4^{2-} increased above detectable concentrations, likely due to pyrite oxidation as confirmed by the depleted sulfur isotope signature of SO_4^{2-} . In the same zone, PCE and trichloroethene (TCE) disappeared and cis-1,2-dichloroethene (cDCE) became the dominant chlorinated ethene. PCE and TCE were likely transformed by reductive dechlorination rather than abiotic reduction by pyrite as indicated by the formation of cDCE and stable carbon isotope data. TCE and cDCE showed carbon isotope trends typical for reductive dechlorination with an initial depletion of ^{13}C in the daughter products followed by an enrichment of ^{13}C as degradation proceeded. At 1000 m downgradient of the source, cDCE was the dominant chlorinated ethene and had reached the source $\delta^{13}\text{C}$ value confirming that cDCE was not affected by abiotic or biotic degradation.

Further downgradient (up to 1900 m), cDCE became enriched in ^{13}C by up to 8‰ demonstrating its further transformation while vinylchloride (VC) concentrations remained low (<1 µg/L) and ethene was not observed. The correlated shift of carbon and chlorine isotope ratios of cDCE by 8 and 3.9‰, respectively, the detection of *Dehalococcoides* sp genes, and strongly reducing conditions in this zone provide strong evidence for reductive dechlorination of cDCE. The significant enrichment of ^{13}C in VC indicates that VC was transformed further, although the mechanism could not be determined. The transformation of cDCE was the rate limiting step as no accumulation of VC occurred. In summary, the study demonstrates that carbon–chlorine isotope analysis and qPCR combined with traditional approaches can be used to gain detailed insight into the processes that control the fate of chlorinated ethenes in large scale plumes.

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1. Introduction

Chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethene (TCE) had been widely used as solvents for more than 50 years. As a result of heavy use of PCE and TCE, many aquifers in the industrially developed world are contaminated by them (Squillace et al., 1999). Due to their complex distribution in the subsurface, engineered remediation of DNAPL contaminated sites is challenging. Hence, natural attenuation of these contaminants in aquifers has gained significant interest as an alternative or complementary remedial strategy (Wiedemeier et al., 1999). Chlorinated ethenes can be degraded by different pathways depending on the redox conditions. During reductive dechlorination, chlorinated ethenes serve as electron acceptors (Holliger et al., 1993). Sequential reductive dechlorination of PCE yields TCE, *cis*-dichloroethene (cDCE), vinyl chloride (VC), and eventually ethene and ethane. As the number of chlorine substituents decreases, the tendency of chlorinated ethenes to become dechlorinated decreases, and consequently more strongly reducing conditions are required (Bradley, 2000). Frequently, cDCE and VC accumulate as a result of incomplete reductive dechlorination caused by an inadequate redox potential, the lack of available electron donor or absence of specific bacteria able to reductively dechlorinate these compounds (Friis et al., 2006a,b). Complete reductive dechlorination of PCE to ethene has been observed in mixed and enriched culture studies (Bradley, 2000; Freedman and Gossett, 1989), but to date the genus *Dehalococcoides* which utilizes H₂ as the electron donor is the only isolated and identified group of microorganisms which dechlorinate cDCE and VC to ethene (Cupples et al., 2003; He et al., 2003; Löffler et al., 2000; Maymo-Gatell et al., 1999). In addition to reductive dechlorination, microbial oxidation of cDCE and VC to CO₂ has been observed under aerobic (Bradley and Chapelle, 1998, 2000b), iron/manganese-reducing conditions (Bradley and Chapelle, 1997; Bradley et al., 1998b; Jennings et al., 2009) and with humic acids as electron acceptor (Bradley et al., 1998a). However, more recent studies demonstrated that low levels of O₂ are sufficient for aerobic VC oxidation (Gossett, 2010) and might also explain VC oxidation in earlier seemingly anoxic microcosms. Fermentative degradation of VC to methane and to CO₂ via acetate under methanogenic conditions has also been reported (Bradley and Chapelle, 2000a). Apart from the microbial degradation pathways, iron-bearing minerals such as pyrite and magnetite are known to abiotically reduce PCE, TCE and cDCE to acetylene via dichloroelimination and VC to ethene by reductive dechlorination (Lee and Batchelor, 2002). Acetylene can be further transformed to acetaldehyde, acetate and ethanol as observed in a laboratory study (Liang et al., 2009).

Although much is known about the degradation pathways of chlorinated ethenes, the field demonstration of their degradation remains challenging especially for cDCE and VC that can be degraded to different products. Recently, new tools have been proposed to identify and quantify chlorinated ethene degradation including compound-specific stable carbon and chlorine isotope analysis (Abe et al., 2009b) and quantitative real-time polymerase chain reaction (qPCR) (Scheutz et al., 2008). The advances in qPCR methods make

it possible to link the detection of specific bacteria to the occurrence of contaminant degradation (Hendrickson et al., 2002; Lu et al., 2006). So far compound-specific isotope analysis has mainly focused on carbon isotopes and it was demonstrated that reductive dechlorination is associated with significant carbon isotope fractionation, especially for cDCE and VC transformation (Hunkeler et al., 1999). Carbon isotope enrichment factor ranging from -14.1 to -29.7% were reported for cDCE and from -21.4 to -30.3% for VC (Hunkeler and Morasch, 2010). Abiotic reductive dechlorination of TCE by pyrite was also shown to cause strong carbon isotope fractionation with a carbon isotope enrichment factor of -21.7% (Liang et al., 2009). Carbon isotope analysis has been used to demonstrate reductive dechlorination in groundwater (Hunkeler et al., 1999; Sherwood Lollar et al., 2001; Imfeld et al., 2008;) and streambed sediments (Abe et al., 2009a). However, when using isotopes to differentiate between degradation pathways, a dual isotope approach is usually preferable because the relative shift of isotope ratios of two elements usually varies depending on the pathway (Elsner et al., 2005). Combined carbon and chlorine isotope analysis has not been applied yet to gain insight into chlorinated ethene degradation at the field scale for several reasons. There is less information available for chlorine isotope fractionation for different degradation pathways than for carbon isotopes. Recent laboratory studies have demonstrated that chlorine isotope fractionation during reductive dechlorination of cDCE and VC is significantly larger than during aerobic oxidation of cDCE and VC, highlighting the potential for using carbon–chlorine isotope analysis to identify reaction pathways (Abe et al., 2009b). However, the magnitude of chlorine isotope fractionation during abiotic transformation of chlorinated ethenes by reduced-iron phases and anaerobic oxidation of cDCE and VC are not known yet. The limited use of chlorine isotope analysis is also due to the difficulty of analyzing chlorine isotope ratios at field relevant concentrations using conventional techniques and due to conceptual complexities in interpretation of chlorine isotope data given the high abundance of the heavy isotope. Recent advances in analytical methods have made it possible to measure chlorine isotope ratios rapidly at field relevant concentrations without complex preparation procedures (Shouakar-Stash et al., 2006). Furthermore, a theoretical framework was developed that demonstrates that chlorine isotope fractionation follows in good approximation to a Rayleigh trend despite the high abundance of the heavy isotopes (Elsner and Hunkeler, 2008). These developments open the possibility to apply a combined carbon–chlorine isotope approach at the field scale.

The main goal of this study was to evaluate the fate of chlorinated ethenes in a large scale plume. In addition to the characterization of contaminant distribution and redox conditions, carbon and chlorine isotope analysis and qPCR were used to gain insight into the contaminant fate. The study provides the first field data for carbon versus chlorine isotope fractionation during degradation of cDCE, which is often the bottleneck for complete degradation of chlorinated ethenes. The investigated plume is situated up to 50 m below the surface and has a length of more than 2 km, which makes analytical tools that provide a maximum of information for each sampling location attractive in order to reduce drilling

costs. Of particular interest was the fate of cDCE and VC that were found at the plume front and whether these new tools make it possible to detect potentially slow but still relevant degradation of cDCE at the site. In addition, the occurrence of abiotic degradation by iron-bearing minerals was evaluated.

2. Study site

The study site is located in the town of Rødékro in southern Jutland, Denmark. The groundwater contamination was caused by a regional dry-cleaning facility which operated from 1967 to 1980. The dry cleaner only used PCE which might however have contained TCE as an impurity. PCE

release occurred due to a leaking underground storage tank and/or leakage from dry-cleaning machines and pipes connecting the tank to the machines and other spills. Hydraulic head data from 55 multilevel monitoring wells and the contaminant distribution indicate that groundwater flows towards the south for approximately 1 km, and then alters its direction to the southeast (Fig. 1).

The geology of the aquifer is illustrated for a section parallel to the groundwater flow direction (Fig. 2). The unconfined aquifer of Quaternary age consists mainly of relatively homogeneous fluvio-glacial sand with occasional presence of gravel, silt and clay lenses (Fig. 2). This part of Jutland aquifers generally contain a very low level of organic

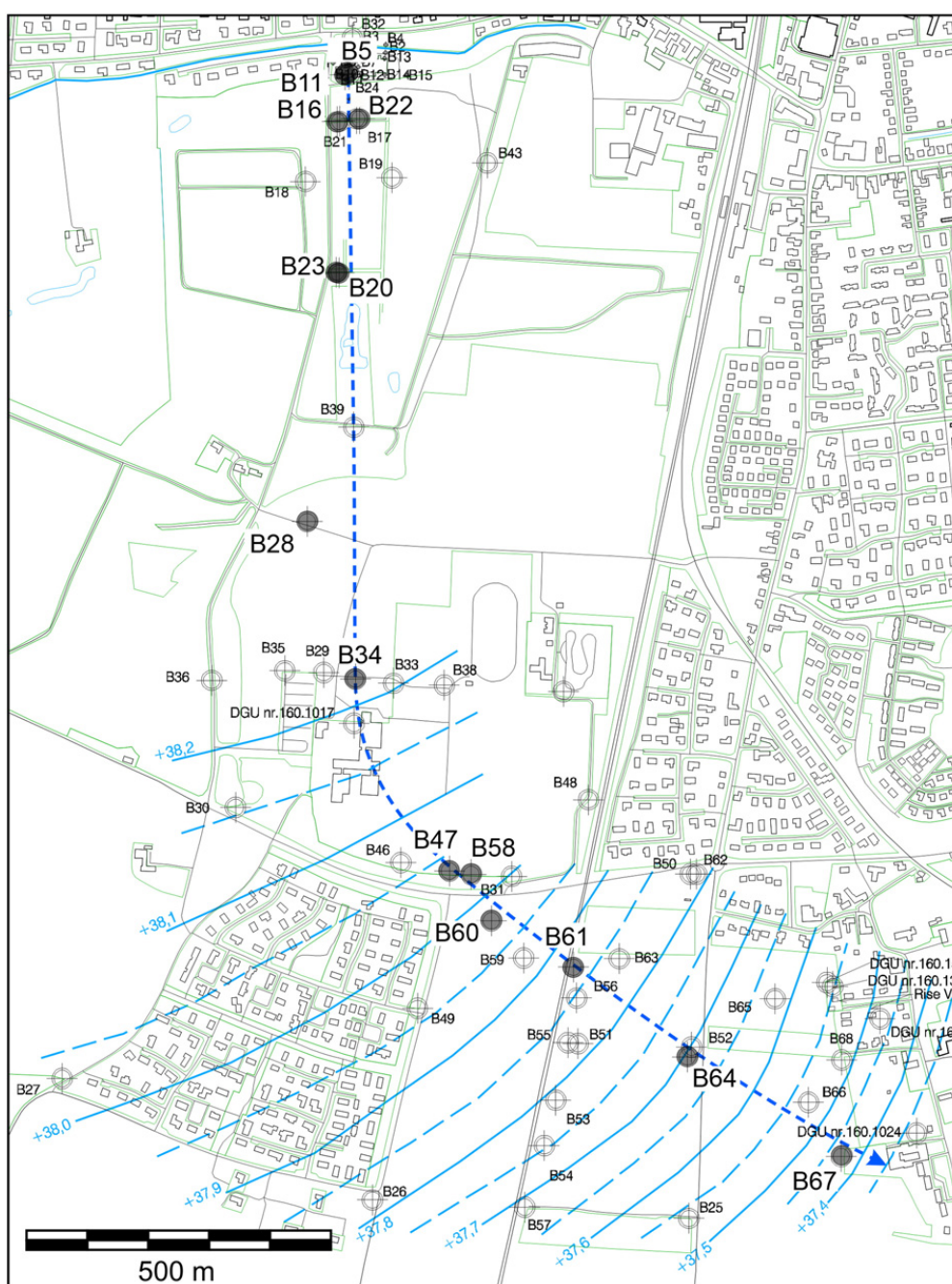


Fig. 1. Monitoring well locations at the site with groundwater equipotential lines (main sampling campaign, February 2006) and approximate flowline along the center of the plume (dashed line). Note that hydraulic heads were only measured for distances >1000 m during the main sampling campaign. The geological section runs along the flowline.

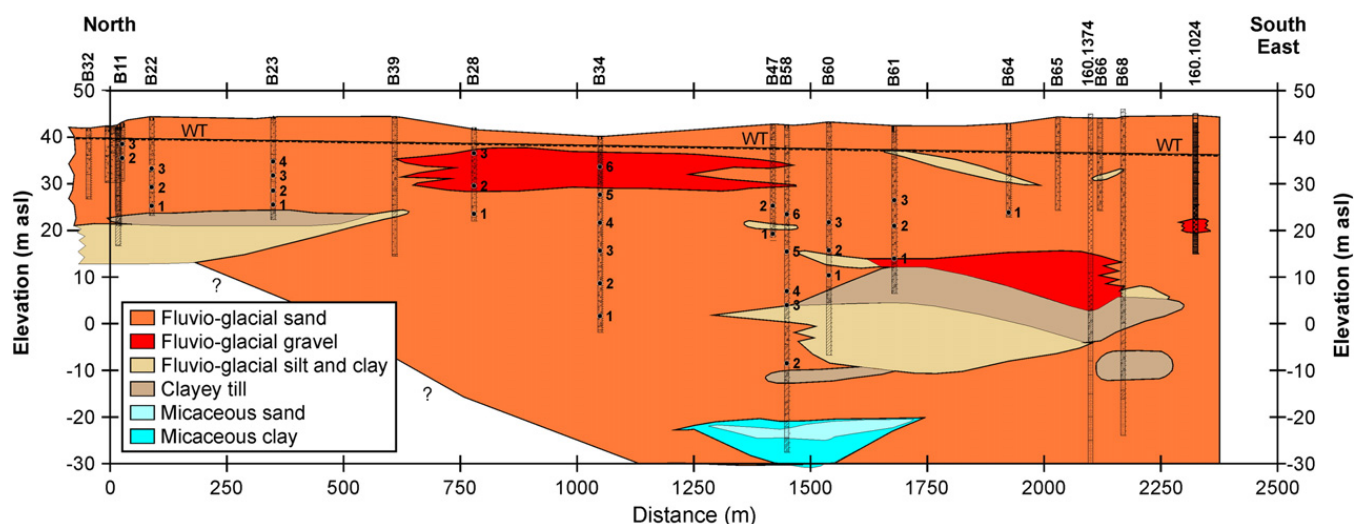


Fig. 2. Geological cross section along a flowline through the center of the plume indicated in Fig. 1. Boreholes near the flowline were projected onto the cross section. Elevations are indicated in m above sea level (m asl). The numbers next to the wells indicate the well number in Table S1. WT: Water table.

matter and significant amounts of iron mainly in the amphibole/pyroxene fraction (Postma et al., 1991; Postma and Brockenhuusschack, 1987). The average hydraulic gradient throughout the study area corresponded to 1.3‰. Based on a pumping test, the local hydraulic conductivity was estimated to be $6.5 \cdot 10^{-4}$ m/s (55 m/day). Using Darcy's law and assuming a porosity of 0.3 typical for sand aquifers in Jutland (Postma et al., 1991), an average groundwater velocity of 0.24 m/day was obtained.

3. Study methods

3.1. Sampling campaigns

The plume characterization took place in several phases due to the large extension of the contamination. Preliminary investigations were carried out during the years 2001 to 2005 concurrently as monitoring wells were installed. A total of 55 multilevel wells with 150 screens were sampled for redox parameters (O_2 , NO_3^- , Mn^{2+} , Fe^{2+} , SO_4^{2-} , CH_4) and for chlorinated ethenes, ethene and ethane concentrations after completing the well installation. Based on these preliminary investigations, 37 screens from 14 multilevel wells located along the core of the plume over a distance of nearly 2 km were selected for a more detailed investigation. These wells were sampled in February 2006 for contaminant concentrations and stable carbon isotope analysis. Samples from several wells were analyzed also for redox sensitive species (NO_3^- , SO_4^{2-} , Fe^{2+} , Mn^{2+} , CH_4) to evaluate the stability of redox conditions at the site by comparing the data with the results from the previous campaigns after well installation. The contaminant concentrations and stable carbon isotope ratios shown in this study originate from this sampling campaign unless otherwise indicated and the sampling campaign is denoted as the main sampling campaign. Since concentrations of redox sensitive species generally varied by less than 30% between sampling campaigns, data sets from the preliminary investigations and the main sampling campaign were combined to characterize the redox conditions in the plume at greater spatial resolution. The results

from the preliminary and main sampling campaigns are summarized in Table S1.

In addition, two complementary smaller sampling campaigns were carried out in September 2006 and April 2007 at a total of 14 screens. The goal of these sampling campaigns was to determine additional parameters that provide insight into the fate of cDCE and VC focusing on the front of the plume where cDCE dominates. The measured parameters include carbon isotope ratios at low concentrations of VC, combined carbon-chlorine isotope analysis of cDCE, concentration measurements of ethene, ethane and acetylene, qPCR of *Dehalococcoides* cells/genes and sulfur isotope ratios of SO_4^{2-} .

3.2. Groundwater sampling

Each monitoring location consists of 1 to 7 PVC pipes of a minimum of 63 mm diameter installed to different depths for multilevel sampling. Each PVC pipe has a screened interval ranging from 1 to 4 m. In general, smaller screened intervals (1 m) were installed in the source and near-source zones and larger screened intervals further downgradient. Groundwater was sampled after purging at least three well volumes of water using a submersible pump placed at 12 m below the ground level. Samples for anion and cation analyses were filtered with a sterile filter (0.45 μ m) and collected in 100 mL polystyrene bottles. Samples for cation analysis were immediately acidified with 1 M nitric acid to pH 2 in the field. For the stable carbon and chlorine isotope analyses of chlorinated ethenes, samples were collected in 40 mL standard VOC glass vials without headspace. For stable carbon isotope analysis of VC at low concentrations, samples were collected in 1-L Schott bottles without headspace. For sulfur isotope analysis, duplicate samples of 500 mL were collected in Schott bottles. Samples for contaminant concentrations and stable carbon/chlorine isotopes were collected without headspace, and stable isotope samples were treated immediately with concentrated NaOH to pH 10 to inactivate microbial activity. Samples for *Dehalococcoides* sp. cell and VC reductase A (*vcrA*) gene analyses were taken in 500 mL Schott bottles without headspace.

3.3. Analyses

Temperature, pH, dissolved oxygen and the electric conductivity of samples were measured on site. Major anion and cation concentrations were measured with an ion chromatograph (DX-120, Dionex, USA) with a detection limit of 0.5 mg/L. Dissolved iron was measured with an ICP-MS method with a detection limit of 10 µg/L in the preliminary and main sampling campaign and colorimetrically (DR/890 Colorimeter, Hach, USA) during the complementary sampling campaigns (detection limit 30 µg/L). Dissolved manganese was determined by flame atomic absorption spectrometry with a detection limit of 5 µg/L. Concentrations of chlorinated ethenes were determined by an accredited commercial laboratory using Purge&Trap GC-MS with detection limits of 0.02 µg/L and relative standard deviations of 9.2% (PCE), 7.5% (TCE), 14% (cDCE) and 7.7% (VC). Ethene and methane was analyzed by the same laboratory using headspace GC-FID (detection limits of 10 µg/L for ethene and methane), except for the complementary sampling campaigns. In the complementary sampling campaigns, the analyses were performed by a different laboratory using headspace GC-FID and additionally included ethane and acetylene concentrations with detection limits of 0.5 µg/L for ethene and ethane, 5 µg/L for acetylene and 10 µg/L for methane. Concentrations of benzene, toluene, ethylbenzene and xylene-isomers (BTEX) were analyzed with a GC-FID after pentane extraction.

Carbon isotope ratios of chlorinated ethenes were analyzed by a GC coupled to an isotope ratio mass spectrometer (DeltaPlus XP, Thermo Scientific, USA) via a combustion interface. The system was equipped with a purge-and-trap concentrator (Velocity XPT, Teledyne Tekmar, USA) connected to a cryogenic trap. A 20-mL sample was purged for 10 min at the purging rate of 40 mL/min and cooled at a cryogenic trap at -140°C . Minimum required concentrations for PCE, TCE, cDCE and VC carbon isotope analysis were approximately 5, 10, 10, and 5 µg/L, respectively. In order to achieve a lower VC detection limit (0.2 µg/L), a 1-L sample was purged for 20 min at the purging rate of 80 mL/min while stirring with a magnetic stirrer, followed by analysis using the same procedure as described above. Chlorine isotope analysis was performed as described previously (Shoukar-Stash et al., 2006). All isotope ratios are reported relative to an international standard (VPDB for carbon, SMOG for chlorine) using the delta notation $\delta = (R/R_{std} - 1) * 1000(\text{‰})$ where R and R_{std} are the isotope ratio of the sample and the standard, respectively.

Dehalococcoides sp. cells and VC reductase A (*vcrA*) genes were quantified using quantitative real-time polymerase chain reaction (qPCR). DNA was extracted from the centrifugation pellet of a 200-mL water sample using the Fast Soil DNA kit (BIO101, Carlsbad, CA) after a modified protocol including a freeze-heat step and extended bead beating as previously described (Baelum et al., 2006). The DNA was diluted and tested for purity by a green fluorescent protein gene test (Lindberg et al., 2007) and found sufficiently clean for the use for qPCR analysis with no inhibition of the reaction. A preliminary PCR analysis for bacterial DNA with general 16S primers confirmed the occurrence of DNA amplification. The presence of *Dehalococcoides* sp. gene was quantified using the primers specific to two sequences in the 16SrRNA gene of *Dehalococcoides* sp. in 16S rRNA (Hendrickson et al., 2002) as well as the *vcrA* gene (Holmes et al., 2006). First, a

determination in the 16SrRNA gene was carried out with the primer pair for the region between 774 and 1212 bp, followed by the more sensitive primer pair for the 1–259 bp region and finally with the *vcrA* primer pair. The quantification limits for *Dehalococcoides* sp. cells with primer 774–1212 and primer 1–259 are $2.2 \cdot 10^6$ and $2.2 \cdot 10^5$ cells/mL sample, respectively, and the quantification limit for *vcrA* is $2.2 \cdot 10^6$ cells/mL.

4. Results and discussion

4.1. Redox conditions

Concentrations of redox sensitive species analyzed in this study (Table S1) are illustrated in Fig. 3. In general, groundwater from less than 12 to 15 m below the surface contained >1 mg/L of O_2 and/or NO_3^- , suggesting oxidizing conditions. At the location with the highest BTEX concentration (B5-3, 7.5 µg/L), dissolved O_2 was <0.5 mg/L and the smallest NO_3^- concentration of the two source zone sampling locations (B5 and B11) was measured (2.1 mg/L) indicating that the BTEX had some effect on the redox conditions. However, the presence of NO_3^- suggests that the BTEX did not create strongly reducing conditions. Groundwater below 15 m depth was deprived of O_2 and NO_3^- and contained dissolved Mn^{2+} , dissolved Fe^{2+} , and elevated SO_4^{2-} concentrations (Fig. 3). The redox conditions may be characterized as manganese/iron reducing in this area (Christensen et al., 2000). The correlation between the disappearance of O_2 and NO_3^- and the increase of SO_4^{2-} and Fe^{2+} concentrations suggests that oxidation of pyrite takes place. Pyrite is known to be present in the sandy aquifers in Jutland (Postma et al., 1991), and pyrite oxidation with O_2 and NO_3^- was previously observed at other field sites (Postma et al., 1991; Schwientek et al., 2008). Sulfur isotopes in SO_4^{2-} were analyzed in samples from several wells (B23-1/3/4, B34-2, and B58-2/4) to further evaluate the origin of the sulfate. The $\delta^{34}\text{S}$ values for SO_4^{2-} varied between -2.6 and -5.0‰ which lies in the typical range of -20 to 0‰ for SO_4^{2-} originating from pyrite oxidation (Clark and Fritz, 1997) and is distinctly different from values for modern marine SO_4^{2-} ($+21\text{‰}$). Hence, the trend towards more reducing conditions with depth is likely influenced by the presence of pyrite in deeper zones of the aquifer, although the pyrite distribution was not determined in the study.

In deeper zones of the aquifer and further downgradient (>1000 m) from the source, slightly higher Fe^{2+} concentrations (>0.5 mg/L) were detected indicating iron reducing conditions (Fig. 3). In addition, traces of methane were detected, despite high SO_4^{2-} levels, which may originate from strongly reducing zones within the clay and silt lenses.

4.2. Concentration evolution along the plume centerline

Fig. 4 shows the distribution of individual chlorinated ethenes in a vertical section along the plume centerline. The complete data set is provided in Table S1. The highest concentrations were detected in B5-3 and B11-3 close to the source. In these wells, PCE concentrations of respectively 96 mg/L (579 µM) and 32 mg/L (193 µM) close to the PCE solubility were measured indicating that PCE DNAPL was likely present close to these wells (Table S1). In these two

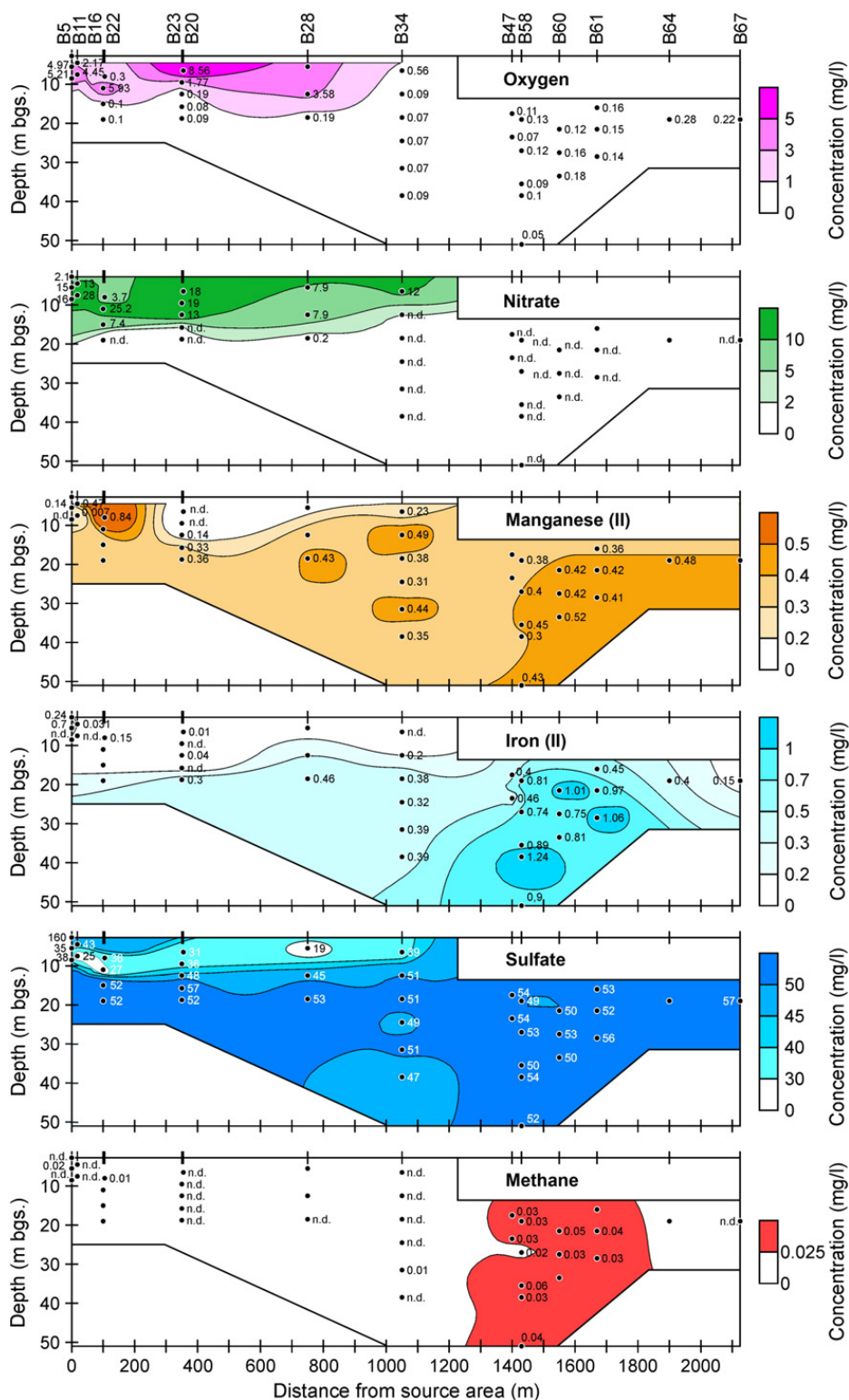


Fig. 3. Concentrations of redox sensitive species in a vertical section along the center of the plume indicated in Fig. 1. The top boundary corresponds approximately to the location of the water table. Depths are indicated in meter below ground surface (m bgs.).

wells, PCE amounts to more than 72% of the total chlorinated ethene concentration on a molar basis with the remainder consisting of TCE, cDCE and traces of VC (<0.1% to total) indicating that biodegradation is moderate in the source zone compared to further downgradient. In the first 400 m, the plume is present in the upper 20 m of the aquifer due to the presence of a clayey zone deeper down. It then plunges and

widens progressively likely due to diverging groundwater flow around a clay zone located further downgradient and groundwater recharge pushing the plume downwards (Fig. 3). As the plume dives to greater depth and migrates to greater distance from the source, it encounters more reducing conditions (see Section 4.1) For sampling points <15 m depth located within 1050 m from the source, PCE was

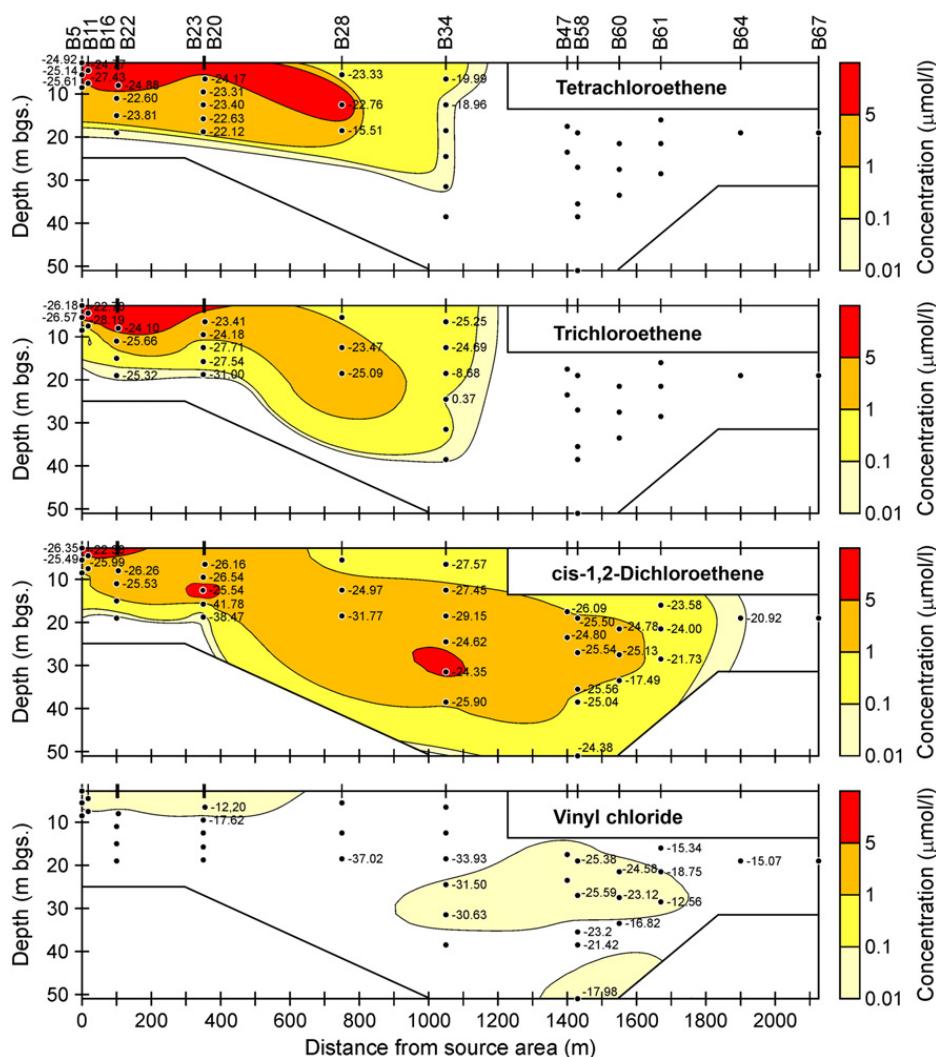


Fig. 4. Concentration contours and stable carbon isotope ratios ($\delta^{13}\text{C}$ ‰) in a vertical section along the center of the plume indicated in Fig. 1. The top boundary corresponds approximately to the location of the water table. Depths are indicated in meter below ground surface (m bgs.).

generally present at the highest concentration of all chlorinated ethenes, while in deeper zones cDCE dominated (Fig. 4). The locations where PCE was no longer dominant generally coincides with the locations where O_2 and NO_3^- concentrations were <0.5 mg/L and the SO_4^{2-} and Fe^{2+} concentrations were elevated (Fig. 2, Table S1). PCE and TCE could be transformed by microbial reductive dechlorination. Once the strong oxidants O_2 and NO_3^- have been consumed, PCE and TCE start to serve as electron acceptors. Alternatively, in the presence of pyrite, PCE and TCE may be transformed abiotically via β -elimination (Lee and Batchelor, 2002). The predominance of cDCE in deeper zones of the site demonstrates that reductive dechlorination of PCE and TCE occurs. Although acetylene was not detected at the site, a possible parallel transformation of PCE and TCE by β -elimination cannot be ruled out as acetylene may be further transformed to acetaldehyde, acetate and ethanol (Liang et al., 2009). Furthermore, the fate of cDCE is not clear based on the concentration data. It could potentially be oxidized coupled to the reduction of manganese (Bradley et al., 1998b). Alternatively, in the more reduced downgradient zone where methane is detected further reductive dechlorination could occur. However, VC is only detected in traces and ethene and

ethane concentrations are below 0.5 $\mu\text{g/L}$ (Table 1). Finally, cDCE may simply be transported further downgradient without any degradation.

4.3. Presence of *Dehalococcoides* sp. cells

The presence of *Dehalococcoides* sp. cells was investigated using qPCR based on water samples. The number of *Dehalococcoides* sp. cells and *vcrA* genes were below the quantification limit of $2.2 \cdot 10^5$ cells/L and $2.2 \cdot 10^6$ genes/L, respectively. However, a signal for *Dehalococcoides* sp. cells was detected with the most sensitive method (primer 1–259) in groundwater from wells B60-1 and B60-3 located close to the front of the plume (at 1550 m from the source, Fig. 4). Most studies report that the presence of *Dehalococcoides* sp. cells coincides with detectable level of VC and ethene (Hendrickson et al., 2002; Lu et al., 2006) and Scheutz et al. (2008) found growth of *Dehalococcoides* containing *vcrA* genes to coincide with an increase in ethene concentrations. A study that compared *Dehalococcoides* sp. densities with cDCE dechlorination rates concluded that rates >0.3 year $^{-1}$ occur where densities are $>1 \cdot 10^7$ cells/L (Lu et al., 2006).

Table 1

Concentrations, stable isotope ratios and detection of *Dehalococcoides* sp. cells in sampling points at the plume front. Sampling took place during the main sampling campaign in Feb. 2006 unless otherwise indicated. –: not measured; +: detected but below the quantification limit. The source $\delta^{13}\text{C}$ value is estimated to be -25.0% . The locations of the wells are given in Fig. 1 and 2.

Well number	Depth m	Isotope balance $\delta^{13}\text{C}$ ‰ VPDB	cDCE			VC		Ethene ^a ug/L	Ethane ^a ug/L	Acetylene ^a ug/L	<i>Dehalococcoides</i> DNA ^a cells/ml
			ug/L	‰ VPDB	$\delta^{13}\text{C}$ ‰ VSMOC	$\delta^{13}\text{C}$					
						ug/L	‰ VPDB				
B34-2 ^b	-31.5	-24.43	560	-24.35 ^c /-23.75 ^d	7.2 ^c /5.7 ^d	0.98	-30.63	<0.5 ^d	<0.5 ^d	<5 ^d	<2.2·10 ⁵
B58-6	-19	-25.50	100.4	-25.50	-	0.62	-25.38 ^c	-	-	-	-
B58-5	-27	-25.54	96.3	-25.54/-25.50 ^d	6.76 ^d	0.70	-25.59 ^c	<0.5 ^d	<0.5 ^d	<5 ^d	-
B58-4	-35.5	-25.56	110.5	-25.56/-25.07 ^d	6.79 ^d	0.56	-23.02 ^c	<0.5 ^d	<0.5 ^d	<5 ^d	-
B58-3	-38.5	-24.99	69.2	-25.04/-	-	0.60	-21.42 ^c	-	-	-	-
B58-2	-51	-24.35	37.1	-24.35/-24.26 ^d	7.19 ^d	0.73	-17.98 ^c	<0.5 ^d	<0.5 ^d	<5 ^d	<2.2·10 ⁵
B60-3	-21.5	-24.78	130.3	-24.78/-25.10 ^d	6.78 ^d	0.90	-24.58 ^c	<0.5 ^d	<0.5 ^d	<5 ^d	+ ^e
B60-2	-27.5	-25.12	230.6	-25.13/-23.73 ^d	7.27 ^d	0.73	-23.12 ^c	<0.5 ^d	<0.5 ^d	<5 ^d	<2.2·10 ⁵
B60-1	-33.5	-16.82	12.1	-/-17.49 ^d	10.61 ^d	0.73	-16.82 ^c	<0.5 ^d	<0.5 ^d	<5 ^d	+ ^e
B61-3	-16	-23.41	18.1	-23.58 ^c /-23.67 ^d	7.76 ^d	0.25	-15.34 ^c	-	-	-	<2.2·10 ⁵
B61-2	-21.5	-23.92	70.2	-24.00 ^c /-24.21 ^d	7.8 ^d /7.5 ^d	0.65	-18.75 ^c	<0.5 ^d	<0.5 ^d	<5 ^d	<2.2·10 ⁵
B61-1	-28.5	-21.01	13.0	-21.73 ^c /-21.48 ^d	9.1 ^d /8.67 ^d	0.72	-12.56 ^c	<0.5 ^d	<0.5 ^d	<5 ^d	<2.2·10 ⁵
B64	-19	-20.51	5.7	-20.92/-20.90 ^c	9.0 ^c	0.27	-15.07 ^c	<0.5 ^d	<0.5 ^d	<5 ^d	-

^a Also measured at B23-1/3/4 but not detected.

^b Depth with highest cDCE concentration.

^c Sampled in Sept. 2006.

^d Sampled in April 2007.

^e Detected but below quantifiable level.

Based on this relationship, no or only slow reductive dechlorination of cDCE is expected to occur at the site.

4.4. Carbon and chlorine evolution along the plume centerline

Carbon isotope ratios ($\delta^{13}\text{C}$) of chlorinated ethenes (Table S1) are illustrated in Fig. 4. In the uppermost screen of well B5 (B5-3) in the source zone, $\delta^{13}\text{C}$ of PCE, TCE and cDCE slightly decreased in the respective order (-24.92 , -26.18 and -26.35%). The small difference among their $\delta^{13}\text{C}$ values might be caused by nearly complete transformation of small amounts of PCE to TCE and to cDCE in some zones accompanied by re-dissolution of PCE from the DNAPL source. Indeed, PCE concentrations close to solubility in B5-3 (96 mg/L or 579 μM) and B11-3 (32 mg/L or 193 μM) confirm that they are located in the source zone. Similar $\delta^{13}\text{C}$ values for PCE, TCE and cDCE in the source zone were previously observed at other field sites (Hunkeler et al., 1999). The concentration-weighted average $\delta^{13}\text{C}$ (denoted as isotope balance) at well B5-3 of -25.03% can be considered to represent the isotope ratio of the PCE source. The average $\delta^{13}\text{C}$ of PCE, TCE and cDCE leaving the source zone (average of B5-1/2/3 and B11-2/3) corresponded to -25.6% , -25.9% and -25.1% , respectively. In the following, the isotope evolution is first discussed for the zone where PCE and TCE disappeared (up to 1050 m) and then for the front of the plume further downgradient.

Between 400 and 1050 m of the plume, PCE became enriched in ^{13}C with increasing depth and distance, demonstrating its transformation (Fig. 4). The TCE became depleted in ^{13}C compared to its source value in deeper zones of B23 (350 m), while it has a similar $\delta^{13}\text{C}$ as the source in B28 (750 m) and finally became enriched in ^{13}C in the two deeper sampling points of B34 (1050 m). The cDCE became also depleted in ^{13}C in the deeper zones of B23 (-41.78 and -38.47%) and B28 (-31.77%). However, its $\delta^{13}\text{C}$ reached a value of around -25% in deeper zones of well B34 where the

highest cDCE concentrations were detected. These trends are consistent with reductive dechlorination of PCE to TCE and cDCE. Reductive dechlorination leads to production of daughter products that are initially depleted in ^{13}C explaining the trends towards more negative $\delta^{13}\text{C}$ of TCE and cDCE. As reductive dechlorination proceeds, the intermediates become enriched in ^{13}C as observed for TCE while the final product approaches the isotope signature of the source. Hence, the carbon isotope data confirm that PCE and TCE are transformed by reductive dechlorination rather than by abiotic reaction with pyrite. In the latter case, TCE and cDCE would not become depleted in ^{13}C compared to the source as these compounds are not produced in significant quantities by abiotic PCE/TCE reduction with pyrite. Furthermore, if PCE and TCE were degraded by β -elimination in addition to reductive dechlorination, the remaining cDCE should become enriched in ^{13}C compared to the source value. Assuming that the isotope enrichment factor for β -elimination of PCE and cDCE are in a similar range as for TCE, the transformation of as little as 5% of the PCE, TCE or cDCE by β -elimination would lead to a shift in $\delta^{13}\text{C}$ larger than 2‰. The good agreement of the $\delta^{13}\text{C}$ of cDCE in deeper zones of B34 (-24.35 to -25.90%) with the source PCE (-25.03%) demonstrates that PCE and TCE are predominantly transformed by reductive dechlorination and that no significant degradation beyond cDCE occurs in the first 1050 m of the plume.

In contrast, at the plume front between B47 (1550 m) and B64 (1900 m), cDCE and VC become enriched in ^{13}C reaching values of up to -17.49 and -12.56% , respectively (Fig. 4). Furthermore, the concentration-weighted average $\delta^{13}\text{C}$ value of all detected chlorinated ethenes shifts from -25.56% in B58 to -16.82% in B60 (Table 1). These trends indicate the loss of carbon from the pool of chlorinated ethene species and that both cDCE and VC are transformed to one or several non-chlorinated products. Transformation may occur by sequential reductive dechlorination. However,

ethene and ethane remained below 1 $\mu\text{g/L}$ at the front of the plume (Table 1), which could indicate that anaerobic oxidation or abiotic degradation may play a role.

4.5. Carbon and chlorine isotope evolution at the plume front

In order to gain additional insight into the degradation mechanism, chlorine isotope ratios of cDCE were measured for wells at the plume front (Table 1). For reductive dechlorination a significant Cl isotope effect occurs since C–Cl bonds are involved in the initial transformation step. For anaerobic oxidation, the isotope effect is not known yet but is expected to be smaller because the initial step likely involves the electron rich C–C bond and hence does not significantly affect chlorine isotope ratios. The chlorine isotope ratio steadily increased at the plume front (Fig. 5) and the shift in $\delta^{37}\text{Cl}$ correlated well with the shift in $\delta^{13}\text{C}$ (Fig. 5). The $\delta^{37}\text{Cl}$ versus $\delta^{13}\text{C}$ shift ($0.48 \pm 0.05\text{‰}$) was larger than in a previous laboratory study ($0.081 \pm 0.004\text{‰}$) with an enrichment culture containing *Dehalococcoides ethenogenes* (Abe et al., 2009b). The stronger chlorine isotope fractionation at the field site could be due to the presence of other cDCE dechlorinating microorganisms or a contribution of abiotic cDCE degradation to acetylene and further products (Lee and Batchelor, 2002). Since reference fractionation factors for abiotic degradation and different cDCE degrading strains are currently lacking, it is not possible to distinguish between these two possibilities. *Dehalococcoides* DNA and traces of methane were detected in the front of the plume indicating conditions under which complete reductive dechlorination can occur (Fig. 3). These observations suggest that reductive dechlorination at least partially contributed to cDCE degradation at the plume front.

The strong enrichment of ^{13}C in VC demonstrates that transformation of VC occurred although ethene and ethane were $< 1 \mu\text{g/L}$. It was not possible to measure $\delta^{37}\text{Cl}$ values for VC due to its low concentrations ($< 1 \mu\text{g/L}$). VC could be transformed by reductive dechlorination, which is associated with strong carbon isotope fractionation, followed by a further

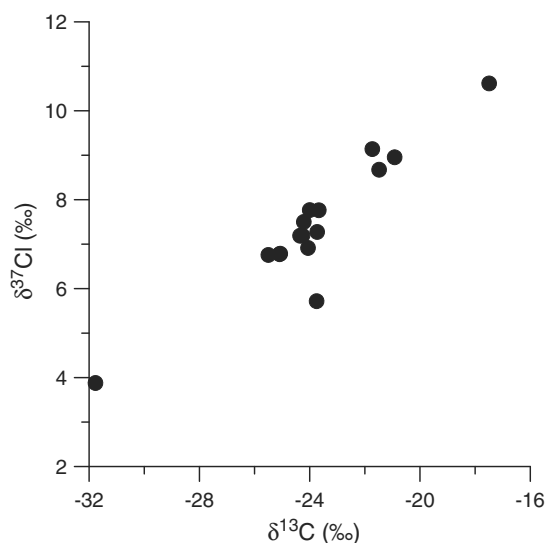


Fig. 5. Correlation of chlorine and carbon isotope ratios of cis-1,2-dichloroethene for wells at the plume front (see Table 1) and wells B23-4 and B28-1.

degradation of ethene as observed in a previous study (Bradley and Chapelle, 2002). Alternatively, VC could be transformed to CO_2 and CH_4 (Bradley and Chapelle, 1999a,b, 2000a). Thermodynamic considerations indicate that the mechanism could involve a highly exergonic reductive dechlorination step (Dolfing, 1999), and thus this pathway would be associated with strong carbon isotope fractionation as well. Hence, while carbon isotope ratios of VC indicate significant degradation of VC, the degradation mechanism cannot be identified unequivocally. VC concentrations remain low likely because VC transformation is faster than VC production. This hypothesis is consistent with the stronger enrichment of ^{13}C in VC than cDCE at the plume front as expected when the consumption of the intermediate (VC) is more rapid than its production (Van Breukelen et al., 2005). Hence, transformation of cDCE is likely the rate limiting step for complete chlorinated ethene degradation at the plume front.

5. Conclusions

At sites with variable redox conditions and the presence of pyrite, such as the one presented in this study, multiple degradation processes can potentially occur including biotic reductive dechlorination, abiotic β -elimination, and biotic oxidation of lower chlorinated by-products under oxic or anoxic conditions. The study indicates that carbon isotopes are suitable for determining the relative importance of reductive dechlorination versus β -elimination for the transformation of the highly chlorinated ethenes. Concentration analysis alone would make it difficult to evaluate the significance of β -elimination as the characteristic degradation product, acetylene, is rapidly transformed further (Liang et al., 2009). The carbon isotope approach relies on the preferential removal of isotopically light chlorinated ethene molecules during β -elimination, which leads to a strong enrichment of ^{13}C in the remaining chlorinated ethene and ethene pool. This isotope enrichment is detected if as little as 5% of the chlorinated ethenes are transformed by β -elimination. Using this approach, it was possible to demonstrate that β -elimination played no significant role in chlorinated ethene transformation in the first 1050 m of the site despite the presence of pyrite. One of the limitations of the approach is that the carbon isotope balance of chlorinated ethenes can, in some instances, shift solely due to the differential retardation of various chlorinated ethenes (Van Breukelen et al., 2005). The combined use of carbon and chlorine isotope analysis could potentially be used to confirm the role of the two processes. However, chlorine versus carbon isotope fractionation during reductive dechlorination and β -elimination are not yet available.

Another key question at sites with chlorinated ethenes is often whether complete or only partial degradation occurs. While reductive dechlorination can usually be evaluated based on the accumulation of intermediate products, it is virtually impossible to demonstrate transformation pathways leading to common compounds such as CO_2 , Cl^- and CH_4 based on concentration analysis. This study clearly demonstrates the value of isotope analysis to assess the degradation of cDCE and VC at the plume front. While a shift in the carbon isotope ratio clearly indicated transformation, it remains difficult to identify the degradation mechanism, which is a

prerequisite to quantify degradation based on isotope data. The use of combined carbon–chlorine isotope analysis and the detection of specific microorganisms at the functional gene level can provide additional insight into degradation mechanisms. In this study, both carbon/chlorine isotope analysis, suggesting cleavage of C–Cl bonds, and qPCR, demonstrating the presence of *Dehalococcoides* sp., are consistent with the occurrence of reductive dechlorination at the plume front. However, the utilized qPCR approach will only reveal known microorganisms and hence provides an incomplete picture of the microbial processes occurring at the plume front. Once the degradation processes are identified, carbon isotope data can be evaluated in a quantitative manner. Assuming that cDCE is transformed by reductive dechlorination and taking into account the range of isotope enrichment factors observed for reductive dechlorination of cDCE (−14.1 to −29.7‰, (Hunkeler and Morasch, 2010)), a detectable shift in $\delta^{13}\text{C}$ (2‰) is expected after 6–13% of the cDCE was degraded. Hence the isotope method is very sensitive to detect cDCE degradation. For VC, carbon isotope enrichment factors are even larger and hence less degradation is needed for a noticeable $\delta^{13}\text{C}$ shift.

In summary, the study demonstrates that the combination of stable isotope analysis and methods of molecular microbiology has a high potential to identify and also quantify degradation processes at chlorinated ethene contaminated sites. The methods are currently limited by the lack of reference values for chlorine isotope fractionation and the lack of knowledge of some of the microorganisms that are involved in the degradation process.

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