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Evaluating the fate of chlorinated ethenes in streambed sediments by combining stable isotope, geochemical and microbial methods

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ABSTRACT

The occurrence of chlorinated ethene transformation in a streambed was investigated using concentration and carbon isotope data from water samples taken at different locations and depths within a 15 × 25 m study area across which a tetrachloroethene (PCE) plume discharges. Furthermore, it was evaluated how the degree of transformation is related to groundwater discharge rates, redox conditions, solid organic matter content (SOM) and microbial factors. Groundwater discharge rates were quantified based on streambed temperatures, and redox conditions using concentrations of dissolved redox-sensitive species. The degree of chlorinated ethene transformation was highly variable in space from no transformation to transformation beyond ethene. Complete reductive dechlorination to ethane and ethene occurred at locations with at least sulfate-reducing conditions and with a residence time in the samples streambed zone (80 cm depth) of at least 10 days. Among these locations, *Dehalococcoides* was detected using a PCR method where SOM contents were >2% w/w and where transformation proceeded beyond ethene. However, it was not detected at locations with low SOM, which may cause an insufficient H_2 supply to sustain a detectably dense *Dehalococcoides* population. Additionally, it is possible that other organisms are responsible for the biodegradation. A microcosm study with streambed sediments demonstrated the potential of VC oxidation throughout the site even at locations without a pre-exposure to VC, consistent with the detection of the epoxyalkane:coenzyme M transferase (EaCoMT) gene involved in the degradation of chlorinated ethenes via epoxidation. In contrast, no aerobic transformation of cDCE in microcosms over a period of 1.5 years was observed. In summary, the study demonstrated that carbon isotope analysis is a sensitive tool to identify the degree of chlorinated ethene transformation even in hydrologically and geochemically complex streambed systems. In addition, it was observed that the degree of transformation is related to redox conditions, which in turn depend on groundwater discharge rates.

1. Introduction

Chlorinated ethenes are among the most frequently detected groundwater contaminants in developed countries (Squillace et al., 1999). Due to their high density and viscosity (Pankow and Cherry, 1996), chlorinated solvents can migrate to a substantial depth in aquifers. Chlorinated solvents have a relatively high solubility compared to regulatory values and low tendency to sorb, facilitating the formation of long contaminant plumes (Schwarzenbach et al., 1983; Wiedemeier et al., 1999) that may reach surface water bodies (Ellis and Rivett, 2007). In the vicinity of rivers and lakes, sediment quality may change abruptly compared to the aquifer, leading

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to different geochemical conditions that influence the fate of chlorinated ethenes (Lorah and Olsen, 1999; Chapman et al., 2007). Higher contents of organic carbon can lead to reducing conditions in stream and lake sediments whereas conditions may become oxic again at the surface of streambeds.

Chlorinated ethenes are relatively oxidized compounds due to the presence of electronegative substituents; therefore, they often serve as electron acceptors with natural or anthropogenic organic compounds as the electron donor species. Tetrachloroethene (PCE) with its four chlorine atoms, undergoes reductive dechlorination under all redox conditions expect in oxic aquifers. Reductive dechlorination of trichloroethene (TCE) occurs under Fe(III)-reducing or more strongly reducing conditions, while reductive dechlorination of cis-1,2-Dichloroethene (cDCE) and vinyl chloride (VC) require sulfate-reducing or methanogenic conditions (Vogel and McCarty, 1985; Vogel et al., 1987; Freedman and Gossett, 1989; DiStefano et al., 1991; Maymo-Gatell et al., 1995; Rügge et al., 1999; Bradley, 2000). Hence, the reductive dechlorination of chlorinated ethenes is strongly influenced by the local redox conditions. The transformation intermediates can also be oxidized metabolically and co-metabolically under oxic and/or anoxic conditions (Wackett and Gibson, 1988; Little et al., 1988; Oldenhuis et al., 1989; Tsien et al., 1989; Nelson et al., 1987; Davis and Carpenter, 1990; Fox et al., 1990; Bradley and Chapelle, 1998; Verce et al., 2001). Since the intermediates, especially VC, have a high toxicity, it is important to know whether complete or only partial transformation is occurring at a site.

Different methods have been proposed to evaluate the occurrence of complete reductive dechlorination of chlorinated ethenes including: 1) characterization of geochemical conditions (availability of electron donors such as H_2, Luijten et al., 2004; assessment of redox conditions, Witt et al., 2002; Lu et al., 2006), 2) characterization of parent and daughter product patterns (Witt et al., 2002) and 3) assessment of the presence and activity of key organisms such as *Dehalococcoides* (Fennell et al., 2001; Hendrickson et al., 2002; Müller et al., 2004; Duhamel and Edwards, 2006; Rahm and Richardson, 2008; Imfeld et al., 2008; Lee et al., 2008). While geochemical conditions and the presence of certain organisms can indicate whether the conditions are favorable for reductive dechlor-ination, these parameters usually do not provide information about the degree of transformation. Furthermore, assessment of concentration patterns can be ambiguous since they are also affected by physical processes such as dilution, especially at the surface-groundwater interface, and because non-unique end products may be formed such as CH_4 and CO_2 (Bradley and Chapelle, 2000). Recently compound-specific isotope analysis was proposed as a complementary tool to assess the degree of reductive dechlorination (Hunkeler et al., 1999; Sherwood Lollar et al., 2001; Hunkeler et al., 2002; Song et al., 2002). The method relies on differences in reaction rates between molecules with light and heavy isotopes, which lead to the enrichment of heavy isotopes in the remaining substrate. Previous studies have suggested that the method is particu-larly sensitive to assess reduction dechlorination of cDCE and

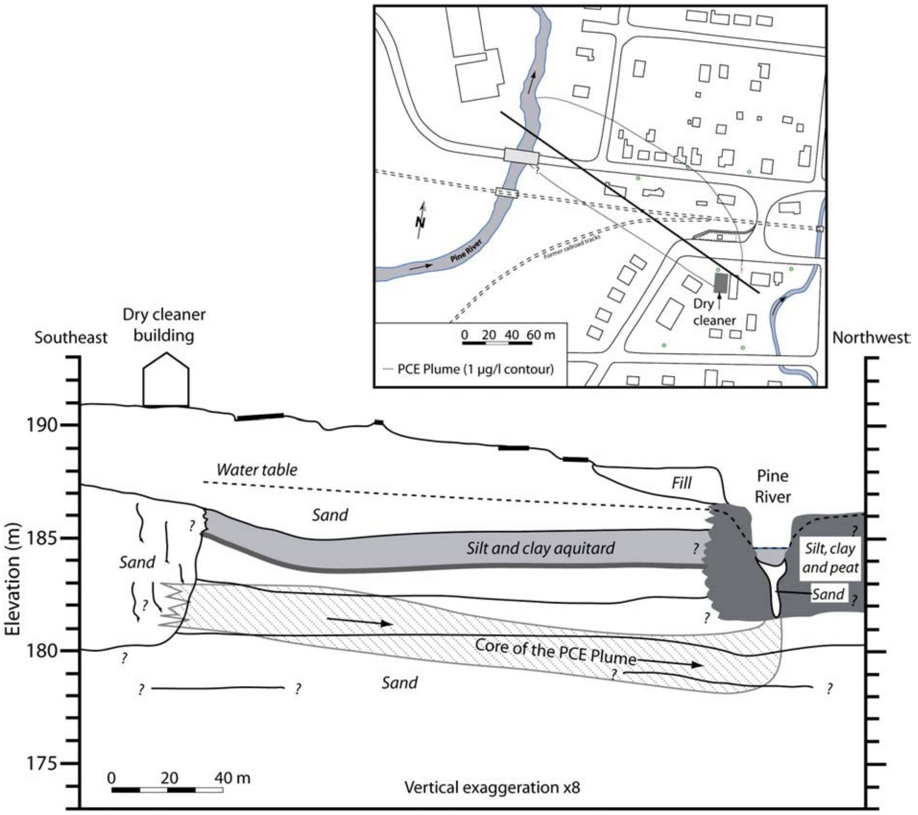

Fig. 1. Schematic illustration of study site with PCE plume migration pathway. Modified from Conant et al. (2004), Hunkeler et al. (2004).

VC because these steps are associated with strong carbon isotope fractionation (Hunkeler et al., 1999; Bloom et al., 2000). Moreover, the isotope approach can potentially distinguish between different enzymatic degradation pathways as they can give rise to different magnitudes of isotope fractionations (Hirschorn et al., 2004; Abe et al., 2009).

The objectives of the study were: 1) to evaluate if stable carbon isotope analysis can be used to document the progress of reductive dechlorination at a groundwater–surface water interface with highly variable hydrological and geochemical conditions, 2) to identify key factors (hydrological, geochemical and microbial) that control the degree of transformation, and 3) to investigate the potential for aerobic oxidation of intermediate compounds at the streambed surface. The study focused particularly on the intermediates, cDCE and VC because their fate critically determines whether or not natural attenuation is successful. The study was carried out at a site where a PCE plume discharges to a river. Water samples were taken in the streambed at high spatial res-olution to assess the degree of chlorinated ethene trans-formation based on concentration and stable carbon isotope analyses. In addition, different factors influencing the occur-rence of complete reductive dechlorination (residence time of water within the streambed, availability of electron donors, occurrence of strongly reducing conditions, and presence of *Dehalococcoides*) were investigated by estimating groundwater discharge rates based on streambed temperature, quantifying sedimentary organic matter (SOM) content, characterizing redox conditions based on dissolved species, and carrying out taxon-specific PCR analysis for *Dehalococcoides* using sedimentary DNA samples. Finally, the potential for aerobic oxidation of cDCE and VC at the streambed surface was investigated using microcosms and a PCR-based approach to detect a gene involved in aerobic transformation of chlorinated ethenes.

2. Study site description

The study site is located 80 km northwest of Toronto and 5 km northeast of the Borden hydrogeological research site of the University of Waterloo. The aquifer was contaminated by PCE spills from a dry-cleaner operated from early 1970s until 1994. PCE DNAPL dissolution causes a contaminant plume which travels in a confined aquifer for approximately 195 m until it discharges into the Pine River (Figs. 1 and 2). The riverbank opposite from the contaminated site does not have a detectable level of PCE and its transformation products, indicating that all of the contaminated groundwater discharges into the river (Conant et al., 2004).

A 30-m-wide transect of direct push profiles at the source zone identified four distinct concentration maxima (Guilbeault et al., 2005), and isotope data from another study suggested that the plume consists of PCE from different spill events (Hunkeler et al., 2004). The contaminant plume experiences very little biodegradation during its migration to the river as indicated by low concentrations of PCE transformation products and relatively stable carbon isotope ratios (Hunkeler et al., 2004). Hydrological and contaminant transport processes in the streambed were investigated previously in the PCE plume discharge zone (Conant et al., 2004). The study demonstrated that the low hydraulic conductivity deposits and the geological heterogeneity of the streambed controlled groundwater dis-charge and contaminant distribution patterns. The PCE degra-dation products, TCE, cDCE and VC, were present at elevated concentrations in the streambed; however, it is not clear from the previous studies whether these compounds were further

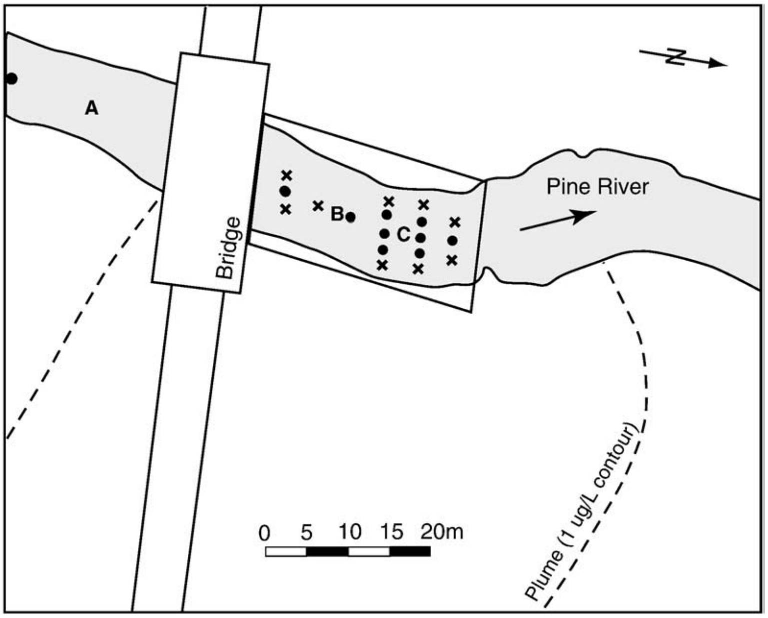

Fig. 2. Plan view with study area. Depth profiles of water and sediment samples were collected from locations indicated by a filled circle while locations where water samples were collected only at the depth of 30 cm are indicated by an x. Locations where sediment cores for the microcosms were sampled are denoted by a letter (A to C).

transformed, neither were the processes responsible for such transformations identified.

3. Study methods

3.1. Streambed temperature measurements

In order to identify the zones of groundwater discharge, streambed temperatures were measured at a depth of 30 cm from the streambed surface throughout a 25 m long stretch of the river in August. All measurement locations are reported with the distance from the northern side of the bridge in flow direction as *x*-coordinate and the distance from the eastern shore as *y*-coordinate. The temperature was measured with a StowAway® TidbiT® temperature logger (Onset Computer Corporation, Pocasset, Massachusetts) which was directly pushed into the streambed. A total of 93 measurements were made during a period of 2 h and compared with a previous study (Conant, 2004). The middle section of the study area was not accessible because a tree had fallen into the river.

The groundwater discharge velocity across the streambed was quantified using an analytical solution to the 1D steady-state heat-diffusion-advection equation (Schmidt et al., 2007):

$$v_z = -\frac{K_{fs}}{\rho_f \cdot c_f \cdot z \cdot n} \cdot ln\frac{T(z) - T_L}{T_0 - T_L} \tag{1}$$

where K_{fs} is the thermal conductivity of the solid–fluid system (site-specific value of 1.45 $\mathrm{J\,s^{-1}\,m^{-1}\,K^{-1}}$ taken from Schmidt et al., 2007), $\rho_f \cdot c_f$ the volumetric heat capacity of the fluid ($4.19 \cdot 10^6\ \mathrm{J\,m^{-3}\,K^{-1}}$), z the depth below the streambed surface and n the porosity (site-specific values between 0.45–0.56 taken from Conant, 2004). Although the surface water temperature varied by 4.6 °C on the day of measurement, quasi-steady state conditions can be assumed at the measurement depth of 30 cm as indicated by numerical simulation of the effect of diurnal temperature variations and the surface water temperature can be approximated by the average temperature (Schmidt et al., 2007). An average groundwater temperature T_L of 9.8 °C (Hunkeler et al., 2004) and the average surface water temperature T_0 for the measurement day of 18.1 °C were used. The calculated velocities have to be considered as approximate values since the groundwater flow direction in the streambed will not everywhere be exactly vertical.

3.2. Streambed water sampling

The sampling locations were selected based on the streambed temperature map and the information from a previous study on the contaminant distribution (Conant et al., 2004). In particular, zones with elevated cDCE and VC concentrations were targeted as well as a zone with high PCE discharge for comparison. At 18 locations within the study area and one upgradient location, water samples were taken at a depth of 30 cm to evaluate the spatial distribution of the contaminants (Fig. 2). At 9 of the 18 locations, samples were taken at the depths of 10, 30, 50, 70 and 90 cm to obtain vertical profiles (Fig. 2). Water samples from the streambed were taken by manually pushing in a drive-point sampler (Conant et al., 2004) which had a dead volume of 1.5 mL. Water was collected in a connected syringe, and before sampling, approximately 20 mL of water was discarded to rinse the inner tube of the sampler. When taking samples for a vertical profile, the sampler was hammered deeper upon completion of sampling at a shallower depth. A total volume of 200 mL was taken from each location for analyzing alkalinity, dissolved iron, major cations and anions, as well as the concentrations of chlorinated ethenes, concentrations of ethene, ethane and methane, and carbon isotope ratios of chlorinated ethenes and ethene. The withdrawn volume represented an approximate radius of influence of 5 cm assuming a porosity of 0.45; hence, it did not significantly affect adjacent sampling depths. Water samples for anion and cation analyses were filtered with a membrane filter (0.45 μm), and those for cation analysis were immediately acidified with 1 M nitric acid. Triplicate 20-mL samples for concentration and carbon isotope analyses of chlorinated ethenes and ethene/ethane/methane were treated with NaOH to pH 10 for the inactivation of microbial activities. Temperature, pH, conductivity and dissolved oxygen concentrations were measured on-site. For dissolved oxygen concen-trations, a colorimetric method was used (Hach DR/850 Colorimeter, USA). Dissolved sulfide was not measured since only a limited sample volume could be taken without disturbing the concentration profiles.

3.3. Sediment-core sampling

For each of the 9 locations where vertical profiling was carried out, an approximately 90 cm long sediment core was taken. A 2″ diameter aluminum pipe was used with a piston consisting of two double-rubber disks. For core sampling, the piston was always kept at the streambed surface while driving down the aluminum pipe by hammering. After reaching a depth of 90 cm, the pipe was pulled out whereby the piston prevented the sand from sliding out of the tube. Immediately after sampling, the aluminum tube was cut by a tube cutter at different intervals, and the sediment core was sampled at approximately 3, 10, 30 and 70 cm from the top of the core. Each sediment sample was taken in quadruple, preserved in screw-capped plastic tubes, and immediately immersed in liquid nitrogen for solid organic matter (SOM) content analysis and DNA extractions. The left-over sediment samples were kept in zip-lock bags for grain-size analysis.

3.4. Aerobic microcosm study

Sediment for microcosms were collected from A) −10 m upgradient of the bridge without any previous contaminant exposure, B) 10 m downgradient of the bridge with a moderate level of contaminant discharge, and C) 18 m downgradient of the bridge with a high level of contaminant discharge (Fig. 2). Upon sampling, each sediment core of 30 cm was sealed completely from both ends and kept cool until microcosm construction. Two series of nine microcosms (VC and cDCE series) were constructed from three sediment cores with three depths. Microcosms were denoted as A-1 (0 to 10 cm from the surface at location A), A-2 (10 to 20 cm at location A), A-3 (20 to 30 cm at location A), B-1 to 3 and C-1 to 3. Microcosms consisted of approximately 10 g wet sediment and 10 mL minimum salt medium (Hunkeler and Aravena,

2000) in a 60 mL serum bottle capped with a Viton® stopper. VC (>99.5% purity, Fluka, Switzerland) and cDCE (>95% purity, Fluka, Switzerland) was added to each microcosm series at the initial air-phase concentration of 0.5 mM. Control microcosms (one per core) were autoclaved three times and treated with NaN_3 to a final concentration of 1% (wt/wt) in order to stop microbial activity.

Microcosm degradation experiments were performed immediately after the construction of microcosms. Before starting an experiment, all microcosms were left uncapped under the fumehood to equilibrate dissolved oxygen concentrations. During the course of the experiment, microcosms were kept at 21 °C on a rotary shaker to enhance the air–liquid phase equilibrium. Microcosms were monitored every 7 to 20 days for substrate and O_2 concentrations with headspace gas chromatography (GC) analysis using a Carlo Erba GC-FID (currently ThermoFinnigan, USA) and SRI GC-TCD (SRI, USA), respectively. The GC analysis did not detect any reductive dechlorination products (ethene and ethane) during the experiments. The aqueous O_2 concentration was calculated from the headspace concentration and kept above 2 mg/L to assure oxic conditions. The microcosms were incubated for one and a half years.

3.5. Analytical and data evaluation methods

Major anion, nitrite and cation concentrations were determined by ion chromatography (Dionex DX-120, USA). Detection limits for anions and cations were 0.1 mg/L. Dissolved iron concentrations were measured colorimetrically using the phenanthroline method with a detection limit of 0.05 mg/L. Chlorinated ethene concentrations were analyzed with a HP-6890 gas chromatograph 6890 equipped with a HP-7694 head space autosampler (Agilent, Palo Alto, USA) and an electron capture detector. After headspace equilibration, a Shimadzu MNZ-1 GC (Shimadzu, Japan) was used for ethene, ethane and methane concentration analyses. Detection limits were 0.9 (PCE), 0.6 (TCE), 1.9 (cDCE), 1.0 (VC), 0.5 (ethene), 0.5 (ethane) and 0.4 (methane) μg/L. Carbon isotope ratios of chlorinated ethenes were analyzed by a GC coupled to an isotope-ratio mass spectrometer with a combustion interface (Thermo Finnigan, Germany). The system was equipped with a purge-and-trap concentrator (Tekmar, Velocity XPT, USA) connected to the GC via a cryogenic trap for chlorinated ethene analysis and an injection loop for ethene analysis in headspace samples. Minimum concentrations for PCE, TCE, cDCE, VC and ethene isotope analysis were approximately 5, 10, 10, 5 and 10 μg/L, respectively. All isotope ratios are reported relative to VPDB using the delta notation given by $\delta^{13}C = (R/R_{std} - 1) * 1000$ (‰) where R and R_{std} are the isotope ratio of the sample and the VPDB standard, respectively.

Based on the carbon isotope ratio, the first order rate constant k of a compound that is only subject to consumption can be estimated as follows (Hunkeler et al., 2002):

$$\Delta\delta^{13}C = \varepsilon \cdot k \cdot t \tag{2}$$

with $\Delta\delta^{13}C$ is the shift in the isotope ratio, ε the isotopic enrichment factor associated with the processes and t the residence time of the compound.

The SOM content of sediment samples was analyzed in dried and ground samples of approximately 100 mg weight using a Rock-Eval 6 pyrolysis instrument with standard cycles (Behar et al., 2001). The grain size distribution of sediment samples were analyzed using a standard sand shaker with sieve sizes 18 to 120 ASTM. The reported uniformity coefficient (UC) was determined, which corre-sponds to the ratio between the grain size that has 60%of the material finer than itself, to the grain size that has 10% finer than itself. Based on measured concentrations of NO_3^-, NO_2^-, Fe^{2+}, SO_4^{2-} and CH_4, dominant redox con-ditions were assigned to the profiles using the criteria defined in Table 1 to simplify the comparison of redox conditions with other parameters. The identification is based on the appearance of the reduced species, except for sulfate reduction, which was identified based on a SO_4^{2-} concentration decrease of at least 20% due to the lack of sulfide data.

3.6. Molecular biology methods

Sediment samples from the streambed and microcosms were preserved at −80 °C until further processing. DNA was extracted with the FastDNA SPIN kit (Bio101) and a FastPrep bead-beating machine (Bio101). PCR reactions were carried out targeting the V3 region on the 16S rDNA gene (≈200 bp) by using primers Eub338f and Eub518r (Ovreas et al., 1997) to test whether the extracted DNA is amplifiable. All DNA samples were PCR-amplifiable as positive results were obtained by PCR with these primers. The *Dehalococcoides* specific primers Fp DGC 774 and Rp DHC 1212 were used to substantiate anaerobic VC dechlorination (Hendrickson et al., 2002). The same PCR conditions as in the original works were used (Hendrickson et al., 2002; Coleman and Spain, 2003a). The DNA samples were also subjected to PCR with specific primers for the EaCoMT gene (CoM-F1L and CoM-R2E) which codes for the enzyme epoxyalkane:coenzyme M transferase that is involved in the aerobic degradation of VC and ethene (Coleman and Spain, 2003b; Danko et al., 2006). DNA from *Nocardioides* sp JS 614 (Coleman et al., 2002) and from the KB-1 mixed culture (SiREM, Guelph, ON, Canada) was used as positive control for the EaCoMT gene and *Dehalococcoides*, respectively. In order to increase the sensitivity of molecular detection, the PCR amplification process was repeated twice whereby a ten-fold dilution of the previous PCR product was used as template for the following PCR with the same primer set. PCR products were visualized on 1.3% agarose gels (Eurobio, France) after ethidium bromide staining. The relative intensity of PCR products on an electrophoresis gel

Table 1
Criteria used for assigning redox conditions.

Compound					
Redox conditions	NO_3^-	NO_2^-	Fe^{2+}	SO_4^{2-}	CH_4
NO_3^--reducing	>1 mg/L	>0.1 mg/L	<1 mg/L	–	<1 mg/L
Fe^{3+}-reducing	<1 mg/L	–	>1 mg/L	–	<1 mg/L
SO_4^{2-}-reducing	<1 mg/L	–	–	> −20%	<1 mg/L
Methanogenic	<1 mg/L	–	–	<1 mg/L	>1 mg/L

–: no criteria defined.

was qualitatively classified from weak (+) to strong (+++) as shown in Fig. 3.

4. Results and discussion

4.1. Characterization of hydrological conditions and sediment composition

The measured streambed temperatures at a depth of 30 cm varied between 12.2 and 16.8 °C. A colder streambed temperature represents zones of faster groundwater discharge whereas a warmer streambed temperature indicates slow discharge or stagnant conditions. The observed temperature range corresponds well with a previous study (Conant, 2004), suggesting stable flow condition at the site over time. Within the study area, two zones with relatively high discharge rates can be identified at 4 and 22 m, respectively from the reference bridge (Fig. 4). The groundwater discharge velocities esti-mated using Eq. (1) varied between 0.04 and 0.29 m/d. This corresponds to a groundwater residence time in the streambed between the deepest (90 cm) and most shallow sampling point (10 cm) of 2.8 to 19.3 days.

Sediment composition analysis demonstrates that sand was the main component of the streambed (Table 2). However, at some locations (16-4, 16-8, 20-4, 20-6 and 24-6), clay, silt and gravel were present at several depths. The SOM content was generally <1% w/w except for the deepest points at 16-4 and 20-4 where a silt layer was present (Table 2).

4.2. Geochemical characterization

Concentrations of redox sensitive species are reported in Table 2. The results show that in general O_2 concentrations remained low at all locations and depths, revealing that this part of the streambed was under anoxic conditions. In the following, the sampling locations are discussed in order of decreasing redox potential. The redox conditions were assigned according to the criteria defined in Table 1.

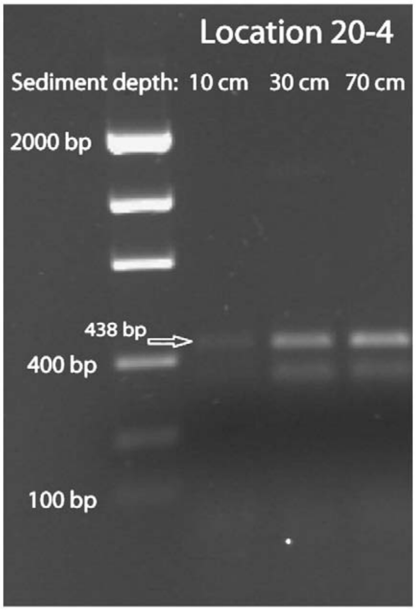

Fig. 3. PCR results with *Dehalococcoides* specific primers Fp DGC 774 and Rp DHC 1212. From left: Low DNA mass ladder, samples from location 20-4 at 10 cm, 30 cm, and 70 cm. The relative intensities of the three samples were used as a semi-quantitative scale ranging from weak (+) to strong (+++).

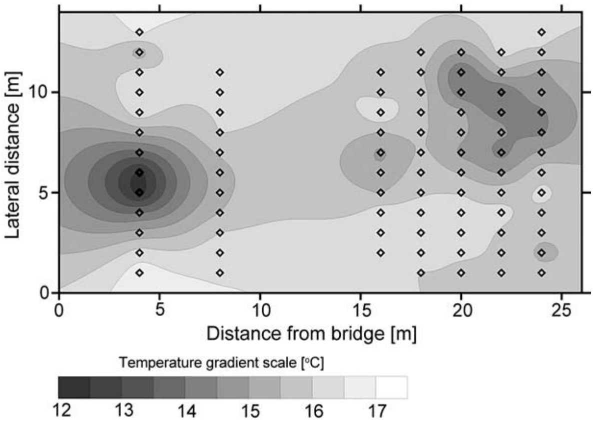

Fig. 4. Streambed temperature map of the studied area at the depth of 30 cm. Symbols indicate the locations with temperature measurements. The x-coordinate corresponds to the distance from the northern side of the bridge, the y-coordinate to the distance from the eastern shore (Fig. 2).

At location 4-6 situated in a high discharge zone (Fig. 4), the highest NO_3^- concentrations were detected with values of up to 10.2 mg/L and nitrite concentrations above 0.1 mg/L suggesting NO_3^- reducing conditions (Table 2). Although SO_4^{2-} concentration decreased towards the sediment surface, SO_4^{2-} reduction is unlikely at this location given the presence of NO_3^-. At the high discharge zone, groundwater flow lines likely converge and if the chemistry varies between different flow lines in the aquifer, steep lateral concentration gradients will occur in the discharge zone. Correspondingly, if the concen-tration profile is not exactly aligned along a flow line, concentration changes with distance will be observed that are not due to reactive processes. This phenomenon also explains why the electrical conductivity decreased towards the streambed and some anomalous isotope trends occurred as discussed later.

At 16-6, NO_3^- concentrations were <1 mg/L and SO_4^{2-} concentrations stable, while the Fe^{2+} concentrations increased indicating Fe-reducing conditions. At 20-6, SO_4^{2-} concentra-tions decreased while CH_4 concentrations remained low suggesting SO_4^{2-} reducing conditions. Surprisingly, in the lowest depth, SO_4^{2-} was absent although it is present further upward. The abrupt drop in SO_4^{2-} concentrations might be caused by stagnant condition at the lowest depth and bypass flow due to the presence of low permeability sediments. Several locations (16-4, 16-8, and 20-4) were intermediate between SO_4^{2-} reducing and methanogenic conditions as indicated by decreasing SO_4^{2-} and increasing CH_4 concentrations. At all of these locations sand/silt was present and at two locations (16-4 and 20-4) the SOM content was high. Finally at two locations (20-8 and 24-6), SO_4^{2-} concentrations were low (<1.06 mg/L) throughout the profile. However, only at 20-8 methanogenesis was observed while CH_4 concentrations remained low (<0.05 mg/L) at location 24-6.

Table 2
Sediment composition, uniformity coefficient (UC), solid organic matter (SOM) content and the PCR responses (for scale see Fig. 3).

		Solid phase[a]					Aqueous phase																	
Location x-y (m)	z (cm)	Lithology	UC	SOM %w	Dehalococ. DNA	EaCoMT DNA	Cond uS/cm	O_2 mg/L	NO_3^- mg/L	NO_2^- mg/L	Fe^{2+} mg/L	SO_4^{2-} mg/L	CH_4 mg/L	PCE ug/L	‰	TCE ug/L	$\delta^{13}C$ (‰)	cDCE ug/L	$\delta^{13}C$ (‰)	VC ug/L	$\delta^{13}C$ (‰)	Ethene ug/L	$\delta^{13}C$ (‰)	Ethane ug/L
4-6	10	Sand	1.93	0.06	−	+++	730	0.37	8.39	0.36	0.05	15.0	0.01	44.6	−30.85	6.7	−	<1.9		<1.0		<0.5		<0.5
	30	Sand	2.09	0.11	−	+++	722	0.31	8.71	0.52	<0.05	14.8	<0.001	43.0	−31.14	6.9	−	<1.9		<1.0		<0.5		<0.5
	50	Sand	2.12	0.06	−	−	727	0.39	9.26	0.40	<0.05	15.6	<0.001	56.5	−30.66	6.8	−	<1.9		<1.0		<0.5		<0.5
	70	Sand	2.32	0.88	−	+++	770	0.30	10.19	0.64	0.43	18.5	0.01	120	−29.42	7.8	−	2.1		<1.0		<0.5		<0.5
	90						871	<0.1	7.24	0.67	0.25	31.3	0.01	338	−27.26	17.4	−32.2	31.5	−28.1	<1.0		<0.5		<0.5
2-6	10	Sand		0.05	−	++	471	0.24	0.47	<0.1		18.8	0.07	<0.9		<0.6		<1.9		<1.0		<0.5		<0.5
	30	Sand	1.88	0.02	−	++	466	0.53	1.43	<0.1		18.8	0.02	<0.9		<0.6		<1.9		<1.0		<0.5		<0.5
	50	Sand	1.72	0.08	−	++	749	0.10	<0.1	<0.1		18.5	0.03	31.3	−29.47	9.0	−30.7	23.4	−29.4	<1.0		<0.5		1.2
	70	Sand	1.90	0.07	−	−	756	0.20	0.22	<0.1		20.3	0.01	54.3	−28.79	8.1	−28.6	10.7	−28.8	<1.0		<0.5		<0.5
16-4	10	Sand	1.81	0.09	−	+++	1045	0.30	−	<0.1	1.93	<0.1	15.55	<0.9		<0.6		<1.9		<1.0		15	−12.8	40
	30						1075	0.26	1.45	<0.1	2.68	0.62	18.75	<0.9		<0.6		<1.9		<1.0		22	−10.6	58
	50	Sand/silt	2.64	0.13	−	−	1036	0.18	0.39	<0.1	1.65	1.08	16.73	<0.9		<0.6		<1.9		<1.0		1.4	−	64
	70	Sand/silt	2.40	11.25	+++	−	884	0.16	0.33	<0.1	3.19	12.3	4.52	<0.9		7.6	−	421	−14.9	102	−28.4	65	−32.7	64
	90						872	<0.1	1.95	<0.1	1.92	7.18	2.03	<0.9		3.3	−	837	−15.5	207	−26.2	479	−26.3	75
16-6	10	Sand	2.15	0.07	−	+++	818	0.28	0.39	<0.1	1.75	35.3	0.31	1.3	−	23.2	14.7	1130	25.5	1.2	−	1.5	−	1.7
	30	Sand	1.87	0.15	−	−	804	0.21	0.31	<0.1	2.05	34.9	0.14	1.1	−	17.3	12.7	778	26.1	<1.0		0.9	−	<0.5
	50	Sand	1.80	0.01	−	+	789	0.24	0.56	<0.1	2.39	34.4	0.14	1.1	−	15.4	−11.1	983	−26.4	<1.0		1.3	−	<0.5
	70	Sand	1.82	0.02	−	−	804	<0.1	<0.1	<0.1	1.84	33.3	0.12	1.5	−	16.6	−12.9	1067	−26.2	<1.0		<0.5		<0.5
	90						814	<0.1	0.43	<0.1	1.28	32.9	0.09	1.8	−	15.7	−12.5	914	−26.3	1.1	−	<0.5		<0.5
16-8	10	Sand	1.99	0.04	−	++	735	0.34	<0.1	<0.1		1.59	10.85	<0.9		<0.6		25	−26.0	<1.0		0.4	−	<0.5
	30						754	0.19	0.34	<0.1		2.76	2.30	<0.9		<0.6		5.7	−	<1.0		<0.5		<0.5
	50	Sand/silt	1.46	0.06	−	−	747	0.29	0.23	<0.1		6.33	2.70	<0.9		<0.6		3.4	−	<1.0		<0.5		<0.5
	70	Sand/silt	1.42	0.04	−	+	774	0.23	0.26	<0.1		5.66	0.06	<0.9		<0.6		<1.9		<1.0		<0.5		<0.5
	90						795	<0.1	<0.1	<0.1		19.9	1.46	<0.9		<0.6		3.1	−	<1.0		<0.5		<0.5
20-4	10	Sand	2.71	0.04	−	++	1264	0.50	<0.1	<0.1		0.31	18.76	<0.9		<0.6		<1.9		<1.0		1.8	−	130
	30	Sand	2.34	0.70	+	−	568	0.90	<0.1	<0.1		0.17	18.91	<0.9		<0.6		<1.9		<1.0		<0.5		386
	50	Sand/silt	2.90	2.35	++	−	447	0.90	0.14	<0.1		0.33	4.01	<0.9		<0.6		<1.9		<1.0		1.8	−	528
	70	Sand/silt	2.88	1.81	+++	−	423	1.00	0.18	<0.1		0.74	6.25	<0.9		<0.6		<1.9		<1.0		<0.5		654
	90						−	1.10	1.00	<0.1		5.03	1.93	<0.9		<0.6		<1.9		<1.0		2.1	−	218
20-6	10	Sand/silt	1.87	0.10	−	++	789		<0.1	<0.1	0.09	17.2	0.26	<0.9		<0.6		15.3	27.6	16.1	5.8	78	−31.3	1.1
	30	Silt/clay	1.56	0.03	−	−	760		<0.1	<0.1	0.05	19.3	0.17	<0.9		<0.6		27.0	25.2	27.9	2.4	71	−32.6	0.5
	50	Silt/clay	2.10	0.03	−	+	767		0.31	<0.1	<0.05	23.8	0.08	<0.9		<0.6		34.2	20.6	30.1	−4.1	51.4	−35.7	<0.5
	70	Gravel/silt	1.55	0.09	−	+	725	0.60	0.78	<0.1	0.09	24.8	0.07	<0.9		<0.6		35.5	18.8	32.4	−6.5	45.6	−40.0	<0.5
	90						727	0.40	<0.1	<0.1	<0.05	<0.1	0.07	<0.9		<0.6		11.2	40.1	25.1	6.8	46	−41.3	<0.5
20-8	10	Sand	1.65	0.13	−	+	832	0.70	6.62	<0.1		0.71	13.44	<0.9		<0.6		<1.9		<1.0		<0.5		5.7
	30	Sand	1.46	0.15	−	++	804	1.30	<0.1	<0.1		0.46	9.15	<0.9		<0.6		<1.9		<1.0		<0.5		4.2
	50	Sand	1.46	0.15	−	++	782	0.78	<0.1	<0.1		0.56	8.85	<0.9		<0.6		<1.9		<1.0		<0.5		<0.5
	70	Sand/silt	1.93	0.11	−	−	775	0.46	<0.1	<0.1		0.97	4.73	<0.9		<0.6		<1.9		<1.0		0.5	−	2.4
	90						761	0.62	0.19	<0.1		<0.1	6.53	<0.9		<0.6		<1.9		<1.0		0.9	−	5.4
24-6	10	Sand/silt	1.99	0.15	−	++	816	0.21	<0.1	<0.1		1.06	0.05	<0.9		<0.6		36.6	−26.7	<1.0		<0.5		<0.5
	30						813	0.19	<0.1	<0.1		<0.1	0.01	<0.9		7.8	−	35.9	−28.7	<1.0		<0.5		<0.5
	50	Sand/silt	2.09		−	+	814	0.20	<0.1	<0.1		<0.1	0.01	<0.9		10.9	−	30.5	−29.3	<1.0		<0.5		<0.5
	70	Sand	1.64	0.08	−	−	846	<0.1	<0.1	<0.1		<0.1	0.01	2.1	−	10.6	−	16.3	−29.5	<1.0		<0.5		<0.5
	90						960	<0.1	<0.1	<0.1		0.66	0.01	1.7	−	9.6	−	6.4	−	<1.0		<0.5		<0.5

Geochemical data and concentrations/isotope ratios of chlorinated ethenes, ethene and ethane in aqueous samples. The locations of the sampling points are given with respect to the northern edge of the bridge in flow direction (*x*-coordinate) and the distance from the eastern shore (*y*-coordinate) in meters. EaCoMT: epoxyalkane:coenzyme M transferase. −: not detected; empty fields: not analysed.

[a] For solid samples, *z* corresponds to the lower end of the samples zone while the upper end corresponds to the *z* of the row above.

4.3. Concentrations and carbon isotope ratios of chlorinated ethenes

Vertical concentration profiles and $\delta^{13}C$ values of PCE and its daughter products were determined at nine locations and summarized in Table 2. The results are discussed below in the order of decreasing redox potential for the five locations where chlorinated ethenes and/or ethene were detected throughout the profile (Fig. 5). The part of the plume discharging between 16 and 24 m from the reference point is likely linked to the main PCE source with a $\delta^{13}C$ of −25.2‰ while the part between 4 and 8 m originates from smaller spills with $\delta^{13}C$ values of −27.5‰ and −32.8‰, respectively (Hunkeler et al., 2004).

Under NO_3^- reducing conditions at 4-6, the PCE concentration decreased while concentrations of transformation products remained low (Fig. 5). In addition, the $\delta^{13}C$ of PCE decreased in flow direction contrary to what would be expected for PCE degradation. If reductive dechlorination of PCE occurred, the $\delta^{13}C$ of PCE would increase in discharge direction. The decreasing $\delta^{13}C$ trend was likely due to the sampling of variable proportions of PCE from different sources with a variable $\delta^{13}C$ migrating along separate flow lines. Indeed, in a previous study the $\delta^{13}C$ of PCE just upgradient from this location varied between −27.7‰ and −30.4‰ due to the presence of multiple PCE sources (Hunkeler et al., 2004).

At 16-6 under Fe-reducing conditions, the highest concentrations of chlorinated ethenes were detected with cDCE as the main compound. The $\delta^{13}C$ of cDCE was substantially more negative compared to that of TCE as expected for the reductive dechlorination of TCE (Bloom et al., 2000). The $\delta^{13}C$ of cDCE remained quite stable in flow direction despite substantial concentration variations, indicating that no further transformation of cDCE occurred at this location. The observed $\delta^{13}C$ of cDCE are similar to the $\delta^{13}C$ value of the main PCE source (−25.2‰; Hunkeler et al., 2004), a typical observation if complete transformation of PCE to a single intermediate compound occurs.

At 20-6 under SO_4^{2-} reducing condition, relatively low concentrations of cDCE, and VC, and higher concentrations ethene were detected (Fig. 5). In the upper four sampling points, the cDCE was highly enriched in ^{13}C and the $\delta^{13}C$ values of cDCE and VC steadily increased demonstrating significant reductive dechlorination of cDCE and VC. The $\delta^{13}C$ of ethene was more negative than that of VC, but approached the $\delta^{13}C$ of the PCE source indicating that ethene was the final degradation product at this location (Hunkeler et al., 1999). In the deepest sampling point, the $\delta^{13}C$ of cDCE and VC were strongly enriched probably due to stagnant and more reducing condition at this depth as already indicated by the absence of SO_4^{2-}. At 24-6, SO_4^{2-} concentrations were low (<1.06 mg/L) suggesting strongly reducing conditions. How-ever, low concentrations of cDCE persisted at this location and the $\delta^{13}C$ of cDCE was approaching the PCE source value (−25.2‰; Hunkeler et al., 2004) indicating that no degrada-tion beyond cDCE occurred.

At 16-4, cDCE, VC and ethene were present in the deepest two sampling points (Fig. 5). While the $\delta^{13}C$ of VC were substantially more negative than those of cDCE as in the location 20-6, the $\delta^{13}C$ of ethene were similar to those of VC, which contradicted the assumption that ethene was the final degradation product. In addition, the $\delta^{13}C$ of ethene was strongly enriched in two uppermost sampling points. These patterns clearly demonstrate that ethene was further degraded throughout the profile. Strong carbon isotope enrichment of ethene due to further transformation was previously observed in a laboratory study (Bloom et al., 2000) but has not been documented at the field scale yet. Ethane

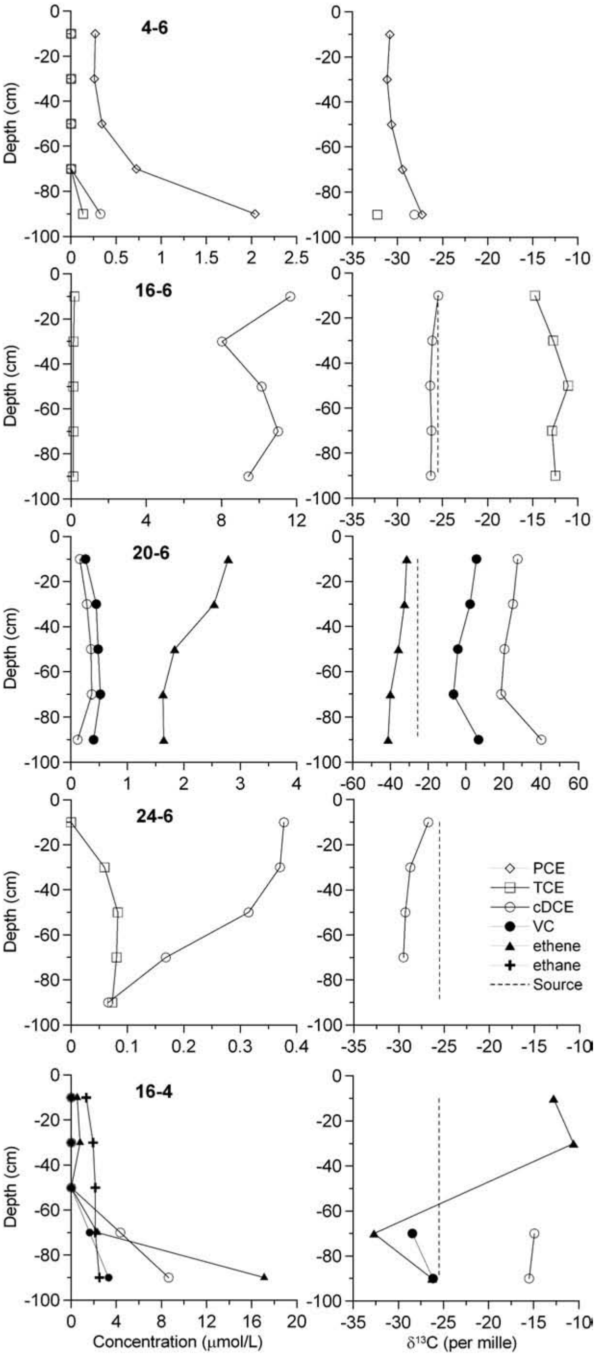

Fig. 5. Depth-variant concentrations and isotope ratios at selected locations. The locations of the sampling points are given with respect to the northern side of the bridge in flow direction (x-coordinate) and the distance from the eastern shore (y-coordinate) in meters.

was a likely transformation product of ethene as it was present throughout the profile. Elevated ethane concentrations (130 to 654 µg/L) were also detected at another location (20-4), where no chlorinated ethenes were present except for some tracers of trans-1,2-DCE (data not shown). However, ethane could potentially also originate from degradation of other organic compounds than ethene under methanogenic conditions (Marrin and Adriany, 1999). The complete degradation of the chlorinated ethenes to ethane and possibly other products at 16-4 and 20-4 probably took place because of the strongly reducing conditions caused by the presence a layer with high SOM content.

At several locations, cDCE is the dominant chlorinated ethene and hence there is an interest to know at what rate the compound is further transformed, if at all. At, 16-6 and 24-6 isotope data clearly indicate that no further transformation of cDCE occurs since the δ^{13}C converges towards the source δ^{13}C value of PCE. At 20-6, a reductive dechlorination rate of 0.05 d^{-1} is obtained based on Eq. (2) for the zone between -70 and -10 cm using a groundwater discharge velocity of 0.068 m/d calculated by Eq. (1), an average isotope enrichment factor of -20.6‰ and taking into account retardation. At 16-4, the rate is larger because cDCE is completely consumed while the residence time is comparable. For comparison, at site bioaug-mented with an enrichment culture containing phylogentic relatives of *Dehalococcoides ethenogenes*, a cDCE reductive dechlorination rate between 2.9 and 4.1 d^{-1} was observed (Morrill et al., 2005).

While carbon isotope data provide insight into the degree and rate of reductive dechlorination, it is not possible to demonstrate VC oxidation in the most superficial zone of the streambed. Since both reductive dechlorination and aerobic oxidation are associated with carbon isotope fractionation, an observed shift could be due to either of the two processes. Furthermore, it might be difficult to sample at the scale where VC oxidation occurs because it would be likely restricted to the uppermost layer. However, the combined carbon and chlorine isotope analysis offers new possibilities to document the role of aerobic oxidation versus reductive dechlorination because the relative shifts in isotope ratios of the two elements are different depending on the pathway (Abe et al., 2009).

4.4. Microbial analyses of the sediment

While all DNA samples were PCR-amplifiable, *Dehalococcoides* was detected only at locations 16-4 and 20-4 (Table 2) characterized by a high SOM and presence of ethane. In 20-4 progressively stronger *Dehalococcoides* signals were detected in deeper sediment sections.

The same DNA samples were also tested for the presence of the EaCoMT gene (Table 2) which is encoding a key enzyme in the aerobic oxidation of VC and ethene (Danko et al., 2006). The EaCoMT gene was detected at all sampling locations including the upgradient sampling location where a previous exposure to the contaminants is not reported. Except for 2 out of 37 locations, the gene was detected in the sandy zone and tended to be present at a higher abundance towards the surface. However, it was also detected in deeper anoxic zones while it was not present where *Dehalococcoides* was detected. The gene may be present in deeper sandy zones because during high river stage, oxygenated water penetrates the sandier zone while the silty zones where *Dehalococcoides* was detected remain continuously anoxic. Alternatively, the bacteria possessing the EaCoMT gene may be transported downward due to mixing of sand during high flow events, and its presence does not necessarily indicate that the oxidation of VC and ethene actively takes place. In order to demonstrate the oxidation capacity by the local microbial community, a microcosm study was carried out using sediment samples (see Section 4.6).

4.5. Comparison of parameters influencing complete reductive dechlorination

In this study, the groundwater residence time within the streambed, the SOM content, redox conditions, and the presence of *Dehalococcoides* were examined as possible factors influencing the degree of reductive dechlorination of chlorinated ethenes (Table 3). The residence time of groundwater within the streambed had a strong influence on the redox conditions. Sulfate reducing and/or methanogenic conditions only occurred in three profiles where the groundwater residence time within the streambed was longer than 10 days (Table 3). In addition, the SOM content played a role. Elevated methane concentrations (>10 mg/L) were only detected in the two profiles (16-4 and 20-4) with a SOM content >2%. In the three strongly reducing profiles, complete reductive dechlor-ination occurred with ethane as final product in the two methanogenic profiles (16-4 and 20-4) and ethene in the sulfate-reducing profile (20-6) (Table 3). *Dehalococcoides* was only detected where methane and ethane were present. In the third profile (20-6), *Dehalococcoides* may be present but below

Table 3
Degree of reductive dechlorination and values for various parameters that potentially influence the degree of reductive dechlorination.

Location	End-product	Velocity calculated using Eq. (1) (m/d)	Groundwater residence time in streambed (d)	SOM (%)	Methane mg/L	Redox conditions	PCR *Dehaloc.*
4-6	PCE	0.26	3.1	0.06–0.88	≤0.01	NO_3^-	No
16-4	Ethane	0.054	14.8	0.09–11.25	2.03–18.75	SO_4^{2-}/Methano	Yes
16-6	cDCE	0.10	8.0	0.01–0.15	0.09–0.31	Fe^{3+}	No
20-4	Ethane	0.046	17.3	0.04–2.35	1.93–18.91	SO_4^{2-}/Methano	Yes
20-6	Ethene	0.068	11.7	0.03–0.1	0.07–0.26	SO_4^{2-}	No
24-6	cDCE	0.057	14.0	0.08–0.15	0.01–0.05	(Methano.)[a]	No

SOM: solid organic matter.

[a] Although conditions were favorable for methanogensis (absence of SO_4^{2-}), no significant methane production was observed.

detection limit. Although the high correlation between presence of *Dehalococcoides* and the occurrence of ethene as a reductive dechlorination product was observed at many sites (Hendrickson et al., 2002; Lu et al., 2006), a recent study reported further potential dechlorinators belonging to the *Chloroflexi* (Kittelmann and Friedrich, 2008). Hence, it may also be possible that other groups of microorganisms were responsible for complete reductive dechlorination to ethane at the location 20-6. In the two profiles with residence times in the streambed of <10 days and lower SOM $\leq 1\%$, redox conditions were less reducing and no or only partial dechlor-ination occurred. Therefore, the degree of reductive dechlor-ination at the studied site was strongly influenced by the redox conditions which were controlled by the residence time of groundwater within the streambed and to a lesser degree by the variability of the SOM content.

4.6. Assessment of cDCE and VC oxidation potential

Although the streambed hydrochemistry indicates anoxic conditions at the studied depths, oxic conditions are likely to occur near the sediment surface due to downwelling of oxygenated water as a function of the streambed topography (Huettel et al., 1998) especially during high river stage. In order to gain additional insight into the potential for VC and cDCE oxidation at the site, microcosm studies were carried out using sediment samples and the initial substrate consumption rates were determined.

While no significant decrease of cDCE with respect to control incubations was detected during 1.5 years of monitoring, VC degradation started immediately after substrate addition (Fig. 6) and followed approximately a first order rate law with half-lives between 8.5 and 13.5 days. Extracted DNA samples from all VC microcosms at the end of the incubation yielded positive responses for the EaCoMT gene. Although sampling location A lies outside of the contaminant discharge zone and has no history of VC exposure, no significant differences among microcosms from the various locations and depths were observed. Hence there is a good agreement between the microcosm study and the EaCoMT gene analysis which both indicate a potential for VC oxidation throughout the site. Rapid mineralization of VC (up to 100% in 8 days) was also observed in a previous microcosm study with material from a streambed in Jacksonville, FL (Bradley and Chapelle, 1998). Moreover, their study also demonstrated rapid mineralization of cDCE; hence, the potential for aerobic cDCE mineralization in streambeds may be more variable between sites than the potential for VC mineralization. When evaluating the potential for VC oxidation it has also to be taken into account that groundwater with VC typically originates from strongly reducing zones and hence different reduced compounds are likely present that also contribute to the oxygen consumption. For example for the sampling point with the highest VC concentration (16-4), 7.8 mg/L of oxygen is required to completed oxidize NO_2^-, Fe^{2+} and CH_4 while VC oxidation corresponded to 2–3% of this oxygen demand. Labile organic compounds such as organic acids from anoxic zones will further contribute to oxygen consumption. Hence, due to the relatively high oxygen demand of anoxic water and because the residence time of the mixed water in the streambed sediment may be short, VC may not become completely oxidized before the groundwater reaches the river.

5. Summary and conclusions

Chlorinated ethene transformation in a streambed was studied by carbon isotope analysis and related to hydrological, geochemical and microbial factors. Stable carbon isotope data allowed determining the degree of reductive transformation by comparing isotope signatures of the intermediates TCE, cDCE, and VC with that of the parent compound PCE. While cDCE accumulated at some locations, dechlorination to ethene or ethane was observed at other locations. Transformation of PCE to ethane was observed at locations with 1) methanogenic conditions, 2) an elevated SOM content and 3) the presence of *Dehalococcoides*. At one location reductive dechlorination to ethene took place without satisfying the above-mentioned criteria. This location was characterized by sulfate-reducing conditions with a low SOM content and *Dehalococcoides* was not detected. The degree of reductive dechlorination was closely related to the local flow regime as highly reducing conditions, a prerequisite to drive reductive dechlorination to completion, required extended residence times of the groundwater within the streambed. Redox conditions and degree of reductive dechlorination varied on a small scale demonstrating the importance of high resolution sampling to demonstrate attenuation of organic contaminants in streambeds.

Due to incomplete reductive dechlorination at most locations, cDCE and VC was detected in the uppermost zone of the streambed sediment in some profiles. Although the potential

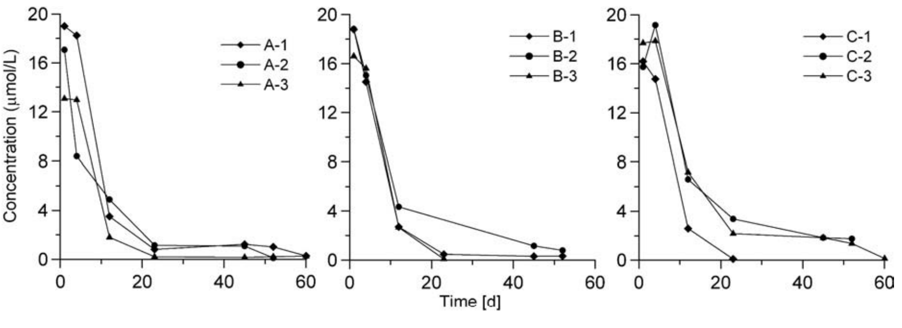

Fig. 6. VC consumption in aerobic laboratory microcosms.

for aerobic degradation of cDCE appears to be small given that cDCE persisted for > 1.5 years in oxic microcosms with material from site, the capacity for VC oxidation seems to be widespread as indicated by the short half-life of 8.5 to 13.5 days in oxic microcosms and the prevalent occurrence of the EaCoMT gene in the study area. However, the residence time within the uppermost layer where oxygen is potentially present may not be sufficient for complete removal of VC before groundwater discharges into the stream especially when other electron donors are present to compete for oxygen.

Regarding methods, the study demonstrated that carbon isotope analysis is a useful method to verify the degree of reductive dechlorination. Especially, it made it possible to demonstrate that ethene is not the final degradation product at the site and that concentration variations at some locations are not due to biodegradation (e.g. cDCE at 16-6). Furthermore, for compounds that are only subject to degradation, first order degradation rate constants can be estimated. However, it is not possible to demonstrate VC oxidation based on the δ^{13}C of VC in the top zone (see above) and it is difficult to quantify rates for compounds that are both subject to production and consumption.

Although DNA analysis only demonstrates the presence of a gene and not the activity of a process, there was in general a good agreement between the detection of the target DNA sequence and the occurrence and the potential of a related transformation process. However, in case of reductive dechlorination, complete reductive dechlorination occurred also at a location where *Dehalococcoides* was not detected, likely due to an insufficient detection limit (a common problem in groundwater studies) or due to the presence of yet unknown microorganisms conducting complete reductive dechlorination. In summary, the study demonstrated that by combining methods that provide information about the general hydrological and geochemical frame, the activity of processes and the presence of specific organisms or genes, detailed insight into natural attenuation processes in complex settings can be gained and limiting factors identified.

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