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Dechlorination after thermal treatment of a TCE-contaminated aquifer: Laboratory experiments

A.K. Friis [a,*,1], E.A. Edwards [b], H.-J. Albrechtsen [a], K.S. Udell [c,2], M. Duhamel [b,3], P.L. Bjerg [a]

[a] *Institute of Environment & Resources, Technical University of Denmark, building 115, Bygningstorvet, DK-2800 Kgs. Lyngby, Denmark*
[b] *Department of Chemical Engineering and Applied Chemistry, University of Toronto, Canada*
[c] *Mechanical Engineering, University of California, Berkeley, USA*

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Abstract

A microcosm study was conducted to evaluate dechlorination of trichloroethene (TCE) to ethene and survival of dechlorinating bacteria after a thermal treatment in order to explore the potential for post-thermal bioremediation. Unamended microcosms containing groundwater and aquifer material from a contaminated site dechlorinated TCE to *cis*-1,2-dichloroethene (*c*DCE), while lactate-amended microcosms dechlorinated TCE to *c*DCE or ethene. A thermal treatment was simulated by heating a sub-set of microcosms to 100 °C for 10 d followed by cooling to 10 °C over 150 d. The heated microcosms demonstrated no dechlorination when unamended. However, when amended with lactate, *c*DCE was produced in 2 out of 6 microcosms within 300 d after heating. Dechlorination of TCE to *c*DCE thus occurred in fewer heated (2 out of 12) than unheated (10 out of 12) microcosms. In unheated microcosms, the presence of dechlorinating microorganisms, including *Dehalococcoides,* was confirmed using nested PCR of 16S rRNA genes. Dechlorinating microorganisms were detected in fewer microcosms after heating, and *Dehalococcoides* were not detected in any microcosms after heating. Dechlorination may therefore be limited after a thermal treatment in areas that have been heated to 100 °C. Thus, inflow of groundwater containing dechlorinating microorganisms and/or bioaugmention may be needed for anaerobic dechlorination to occur after a thermal treatment.

Keywords: Remediation; Dechlorination; Groundwater; Chlorinated ethenes; Thermal treatment

1. Introduction

Perchloroethene (PCE) and trichloroethene (TCE) are widespread groundwater pollutants (WHO, 2004), and their complex spreading pattern in the subsurface challenges remediation technologies (NRC, 1997, 1999). Thermal remediation technologies have been applied successfully at an increasing number of sites (US EPA, 2004). During such treatments the subsurface source zone is commonly heated to temperatures of 100–120 °C, corresponding to pressures of approximately 100–200 kPa (1–2 atmospheres, respectively), to increase contaminant mobility and volatility (Davis, 1997). Nevertheless, residual contamination can remain in the subsurface (Davis, 1997) that may then be removed by less aggressive methods such as bioremediation.

The potential for post-thermal bioremediation depends, in part, on the microbial capabilities after the treatment. Previous results on microbial survival at field sites are inconsistent, demonstrating that the number and activity of microorganisms have increased (Eddy-Dilek et al., 1993; Dettmer, 2002), decreased (Krauter et al., 1995; Balshaw-Biddle et al., 2000), or remained unchanged (Richardson et al., 2002) within six weeks or within six months

* Corresponding author. Tel.: +45 3025 2030; fax: +45 4593 2850.
E-mail address: akf@er.dtu.dk (A.K. Friis).
[1] Present address: Novozymes, Denmark.
[2] Present address: Mechanical Engineering, University of Utah, USA.
[3] Present address: GeoSyntec Consultants, Guelph, Canada.

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(Azadpour-Keeley et al., 2004) after steam injection or electrical resistance heating (ERH). These results were not correlated to a specific thermal technology, nor to a specific analytical method. A laboratory experiment has examined the effect of heating in a closed system by injecting steam in sediment columns (Richardson et al., 2002). This demonstrated decreased microbial activity immediately after heating although pre-steamed levels were reached within 30 d. Fortunately, some microorganisms can survive the elevated temperatures and degrade contaminants including PAHs and hydrocarbons (Krauter et al., 1995; Richardson et al., 2002). However, the potential to degrade chlorinated ethenes after thermal treatments has not been investigated (Dettmer, 2002).

Chlorinated ethenes can be degraded anaerobically in the subsurface by reductive dechlorination. Various microorganisms can dechlorinate PCE and TCE to *cis*-dichloroethene (*c*DCE) (Holliger et al., 1993), whereas only members of the genus *Dehalococcoides* have been demonstrated to reductively dechlorinate *c*DCE to ethene (Maymó-Gatell et al., 1997). Complete dechlorination to ethene is often limited by lack of sufficient electron donors and/or specific dechlorinating microorganisms (Harkness et al., 1999). Knowledge on the survival of dechlorinating microorganisms in subsurface environments after thermal treatments is therefore essential for predicting the potential for post-thermal biodegradation of chlorinated ethenes. Such information can potentially be obtained from field studies, but evaluation of the effects of temperature on the anaerobic dechlorination can be masked due to spatial variability in temperature development, water chemistry and aquifer properties (affecting hydraulics, geochemistry and microbiogy).

In the present study, we characterize the function and capabilities of dechlorinating species following a thermal treatment in a laboratory-based microcosm study under strictly controlled conditions.

In a previous paper, we described changes in redox conditions, pH, alkalinity, and the concentrations of organic matter following treatment in the same experimental system (Friis et al., 2005). These results demonstrated that organic matter was released and redox conditions were maintained in unamended microcosms after heating.

2. Materials and methods

2.1. Site description and sampling

From 1946 to the 1960s, the Fort Lewis East Gate Disposal Yard, WA, USA was used as a disposal site for waste containing TCE, petroleum, oils and lubricants (US Army Corps of Engineers, 2002). It was later converted to a maintenance facility where TCE was used as a degreasing agent from 1963 until the mid-1970s. In 1989, this site was placed on the National Priorities List due to its extensive contamination. The area studied in this research was primarily contaminated with TCE, *c*DCE, and oils. In 2004 the source area was thermally treated using ERH.

Prior to heating, aquifer materials were collected aseptically and anaerobically from three locations: E07 (6 m below surface (mbs), brown/grey sand), F12 (14 mbs, brown sandy gravel) and J10 (9 mbs, grey sandy gravel) and groundwater from one location (B09). Cores were collected with a Rotosonic drill rig and immediately transferred to a sterilized field-portable glovebox with an Ar atmosphere in order to avoid contact with O_2 and introduction of non-indigenous microorganisms. The outer 2–5 cm of the sediment material was removed in the glovebox, and the pristine sediment was transferred into Ar-flushed diffusion-proof aluminium bags with internal Teflon coating. The bags were folded to contain a minimum headspace and sealed with aluminum tape to avoid diffusion of O_2. Strip indicators (Merck® anaerobic indicators) confirmed that the portable glovebox remained anaerobic. Details are given in Friis et al. (2005). Groundwater for microcosms was sampled with a non-H_2-releasing pump (a peristaltic pump, Chapelle et al., 1997) from location B09 at the field site. In addition, groundwater for characterising the water quality at locations E07, F12 and J10 was sampled.

2.2. Microcosm set-up and incubation conditions

Microcosms were constructed in 500 ml sterile glass bottles with 100 g (wet weight) of well-mixed aquifer material, 200 ml of sterile filtered groundwater and sealed with 1 cm butyl rubber stoppers. These microcosms were sampled repeatedly to measure redox chemistry and the concentration of chlorinated solvents. In addition, sacrificial microcosms were constructed in 118 ml sterile bottles with 30 g (wet weight) of well-mixed aquifer material and 30 ml of sterile filtered groundwater. Aquifer material for each location was homogenized by thorough mixing with a spoon in a large container within the glovebox prior to setup of 500 ml and 118 ml microcosms in order to obtain identical initial microbial populations from each sediment location. The different sediment/groundwater/headspace ratios resulted in maximally 8% lower aqueous concentrations of TCE and *c*DCE in sacrificial microcosms compared to microcosms which were sampled repeatedly. All microcosms were set up anaerobically in a Coy glovebox (<5% H_2). Several attempts were made to remove excess H_2 from the anaerobic glovebox: The headspace of each microcosm was flushed for 2–3 min with N_2–CO_2 (80:20) and groundwater was added outside the anaerobic box while flushing groundwater and microcosms with N_2–CO_2.

Aquifer material from each of the three sample locations were used for similar series of heated and unheated microcosms (Fig. 1). One unheated set of microcosms included two unamended, four lactate-amended (5 mM), and two inhibited controls (0.75‰ $HgCl_2$). The heated set of microcosms consisted of three unamended, six lactate-amended

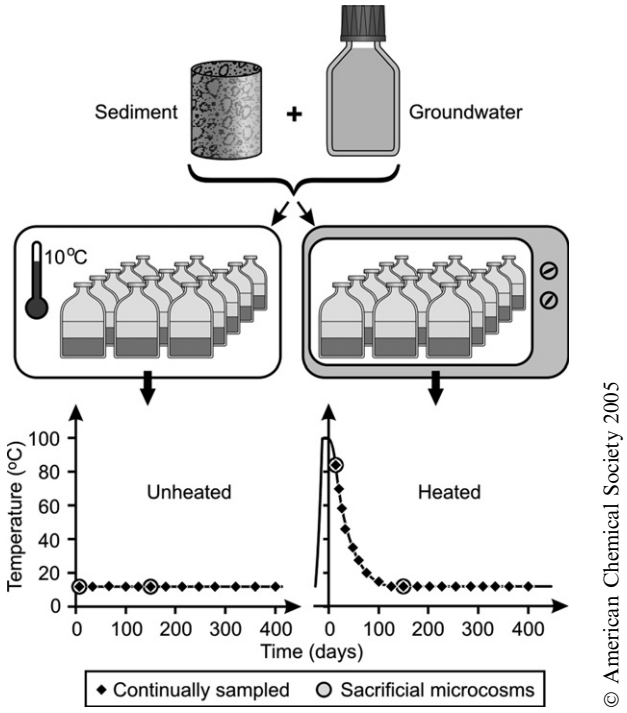

Fig. 1. Experimental setup. Unheated and heated microcosms were either unamended or lactate-amended. Sacrificial microcosms were set up to be able to collect sediment samples used for DNA extractions. Reprinted with permission from Friis et al. (2005).

(5 mM), and three inhibited controls (0.75‰ $HgCl_2$). After 200 d of incubation, the number of microcosms was decreased to one unamended and two lactate-amended for the unheated microcosms, and two unamended and two lactate-amended for the heated microcosms. Also, the frequency of sampling decreased because only limited dechlorination had occurred within the first 200 d. In the inhibited and unamended controls TCE was dechlorinated to *c*DCE (no mass loss) in microcosms with unheated sediment from locations E07 and J10, indicating microbial activity despite $HgCl_2$ addition. The inhibited heated controls did not show any dechlorination or significant abiotic losses taking the uncertainty in temperature dependency of the Henry's law constant into account (data not shown).

As shown in Fig. 1, a thermal treatment was simulated in the heated microcosms by linearly increasing the temperature from 10 to 100 °C over 10 d, maintaining the temperature at 100 °C for 10 d, and subsequently decreasing the temperature exponentially to 10 °C over five months. The temperature was monitored in incubators and followed the expected decrease. The temperature change was slow and based on experience from previous field sites. The slow cooling was also applied because it has been demonstrated that rapid cooling of sediments can decrease microbial survival (Richardson et al., 2002). TCE (20 μM) and lactate (5 mM) were added to the microcosms after heating and before the first sampling. During sampling, microcosms were kept at the given temperature in incubators and water baths. Unheated microcosms were incubated at 10 °C, which was similar to the groundwater temperature at Ft. Lewis of 12 °C (US Army Corps of Engineers, 2002).

2.3. Analytical procedures and chemicals

Chlorinated ethenes were analyzed by GC using an Agilent 6890N gas chromatograph equipped with a mass selective detector (MS, Agilent 5973) and a 25 m × 320 μm × 1 μm (nominal) capillary column (J&W GSQ) with He as a carrier gas. One ml aqueous samples were injected into sealed vials that were heated to 80 °C for headspace gas analysis. Initial content of chlorinated ethenes in the sediment was estimated by transferring 1 g of sediment slurry into rapidly sealed vials which subsequently were analyzed in the same way as the aqueous samples. Standards for *c*DCE, 1,1-dichloroethene (1,1-DCE), *trans*-dichloroethene (*t*DCE), and TCE were prepared volumetrically in aqueous solutions from a concentrated free-phase stock solution. Vinyl chloride (VC) and ethene were added as gases to the headspace vials using a gastight syringe. Ethene appeared as a broad peak on the chromatogram, making low concentrations hard to differentiate from background noise. Detection limits were of 0.02 μM TCE and *c*DCE, 0.07 μM VC and 1.02 μM ethene. Chloroform served as an internal standard. TCE (>99.5% purity, Merck), *c*DCE (>97%, Acros), 1,1-DCE (>99.5%, Fluka), and *t*DCE (>97%, Fluka) were used as calibration standards. VC was of >99.97% purity (Gerling, Holz & Co.) and ethene was obtained as a pure gas (Mikrolab). Standards were prepared for each sampling event and normalized to the sum of TCE and *c*DCE in inhibited microcosms at day 55 for heated microcosms and day 73 for unheated microcosms. The analyses at these days were representative for the analytical variation and the normalization reduced scatter caused by variation in standards and performance of the GC–MS. Samples for analyses of fatty acids were filtered through 0.45 μm nylon filters, acidified with 50 μl 17% H_3PO_4 per ml of sample and kept frozen until analysis by ion chromatography using a Dionex IonPac ICE AS 1 column, DIONEX AMMS ICE II suppressor, and WATERS 432 conductivity detector.

The redox indicators were investigated as described in Friis et al. (2005) with the same sampling frequency as for chlorinated ethenes. Briefly, dissolved Fe (Fe^{2+}) was measured immediately in filtered samples (0.45 μm) using the ferrozine method. Dissolved Mn (Mn_{diss}) was analysed in filtered samples using an atomic absorption spectrometer with flame detection (279.5 nm), and anions (NO_3^-, SO_4^{2-}) were analyzed using ion chromatography. The pH was analyzed within two min of sampling using a microelectrode with temperature correction, and H_2 was analysed using a Trace Analytical RGD2 Reductive Gas Detector GC. CH_4 was analyzed by GC with a flame ionization detector. Headspace concentrations of H_2 and CH_4 were then

converted to aqueous concentrations by using Henry's law with temperature dependency (Wilhelm et al., 1977).

The distribution of chlorinated ethenes in water, headspace, and sediment at elevated temperatures (100 °C) is described by Friis (2006). In these microcosms more than 75% of the TCE, *c*DCE, VC, and ethene mass was in the gaseous phase at 100 °C. In contrast, at 10 °C, the majority of TCE and *c*DCE mass was in the aqueous phase whereas less than 50% of VC and less than 10% of ethene was in the aqueous phase.

The chlorinated ethenes were analyzed in the aqueous phase and reported as an equivalent aqueous concentration (C^{α}). This allows for comparisons with the aqueous concentrations of dissolved electron acceptors (i.e. Fe^{2+}, Mn_{diss} and SO_4^{2-}). C^{α} was estimated as if all mass were in the aqueous phase:

$$C^{\alpha} = \frac{C_w V_w + C_h V_h + C_s m_s}{V_w} \quad (1)$$

where C is the concentration and m is mass of a given compound in h, headspace; w, water; and s, sediment, all given at the time of sampling.

2.4. DNA extraction and nested PCR

Microcosms were sacrificed and sediment slurry was used for DNA extraction and subsequent PCR analyses to investigate the presence of dechlorinating microorganisms at day 10 and 150. Unfortunately, samples obtained after 400 d of incubation were broken during transport from Denmark to Canada and could therefore not be analyzed. Following the manufacturer's recommendations the total DNA from aquifer material (1–1.5 g wet weight) was extracted using the UltraClean Soil DNA Kit (MoBio Laboratories, Inc.). Amplification reactions (20 µl total volume) contained 1 × reaction buffer (New England BioLabs), 0.4 µM of each primer (Table 1), 300 µM of each deoxynucleotide triphosphate (MBI Fermentas), 0.5 U/reaction of AmpliTaq polymerase (New England BioLabs), and 1–3 µl of template DNA. The primers used for amplification were a general bacterial primer pair (Lane, 1991) and four specific primers for known dechlorinating microorganisms (Table 1). Primers for *Dehalococcoides*, *Sulfurospirillum*, *Dehalobacter*, and *Geobacter* were designed to amplify all dechlorinating species in these genera (Duhamel and Edwards, 2006; Grostern and Edwards, 2006). The thermocycling program for the initial amplification with general bacterial primers was: Initial denaturation for 5 min at 94 °C; 35 cycles of 94 °C for 1 min, annealing at 55 °C for 1 min, and 72 °C for 2 min; followed by a final extension at 72 °C for 5 min.

The population of dechlorinating bacteria was too low to be detected from direct PCR with specific primers (data not shown), consequently, a nested PCR approach was taken (Löffler et al., 2000; Fennell et al., 2001). General bacterial primers were used in the first PCR followed by a second PCR with specific primers for certain known dechlorinating genera (Löffler et al., 2000). The second PCR was performed with the amplified product (1 µl) from the initial PCR as templates. The second amplification was performed under the same conditions as above except that the annealing temperature was 52 °C for *Dehalococcoides*,

Table 1
Designations, positions and sequences of primers used in this study for nested PCR

Primer set and sequence	Specificity	Reference
Eubacteria		
27f 5′-AGA GTT TGA TCC TGG CTC AG-3′ 1492r 5′-GGT TAC CTT GTT ACG ACT T-3′		Lane (1991)
Dehalococcoides		
1f 5′-GAT GAA CGC TAG CGG CG-3′ 1386r 5′-CCT CCT TGC GGT TGG CAC ATC-3′	*Dehalococcoides*, *Flexibacter*, *Microscilla* *Dehalococcoides*	Hendrickson et al. (2002) Duhamel et al. (2004)
Geobacter		
73f 5′-CTT GCT CTT TCA TTT AGT GG-3′ 485r 5′-AAG AAA ACC GGG TAT TAA CC-3′	*Trichlorobacter thiogenes*, *Geobacter* strain SZ	Duhamel and Edwards (2006)
Dehalobacter		
477f 5′-GAT TGA CGG TAC CTA ACG AGG-3′ 647r 5′-TAC AGT TTC CAA TGC TTT ACG G-3′	*Dehalobacter restrictus*, *Dehalobacter* strain TCA1	Grostern and Edwards (2006)
Sulfurospirillum		
114f 5′-GCT AAC CTG CCC TTT AGT GG-3′ 421r 5′-GTT TAC ACA CCG AAA TGC GT-3′	*Sulfurospirillum multivorans*, *S. halorespirans* PCE-M2, *S. deleyianum*	Duhamel and Edwards (2006)

59 °C for *Sulfurospirillum* and *Geobacter*, and 62 °C for *Dehalobacter*. The presence of DNA in PCR products was verified by agarose gel electrophoresis alongside a 1 kb DNA Ladder (New England BioLabs).

3. Results and discussion

3.1. Dechlorination in unheated microcosms

*c*DCE was produced in unheated and unamended microcosms with sediment from locations E07 and J10, and TCE was not dechlorinated with sediment from location F12 (Fig. 2). The lack of TCE dechlorination with sediment from location F12 corresponded with *c*DCE and VC concentrations below detection in field samples (Table 3). For locations E07 and J10, the presence of dechlorination products *c*DCE and VC in the field (Table 3) suggest the dechlorination of TCE can occur in these sediments and that *Dehalococcoides* capable of *c*DCE dechlorination were present at this location. The lack of reductive dechlorination of TCE in the microcosmos could be a result of electron donor limitation, a phenomenon which previously has been discussed (e.g. Fennell et al., 1997). As expected, addition of an electron donor (lactate) resulted in further

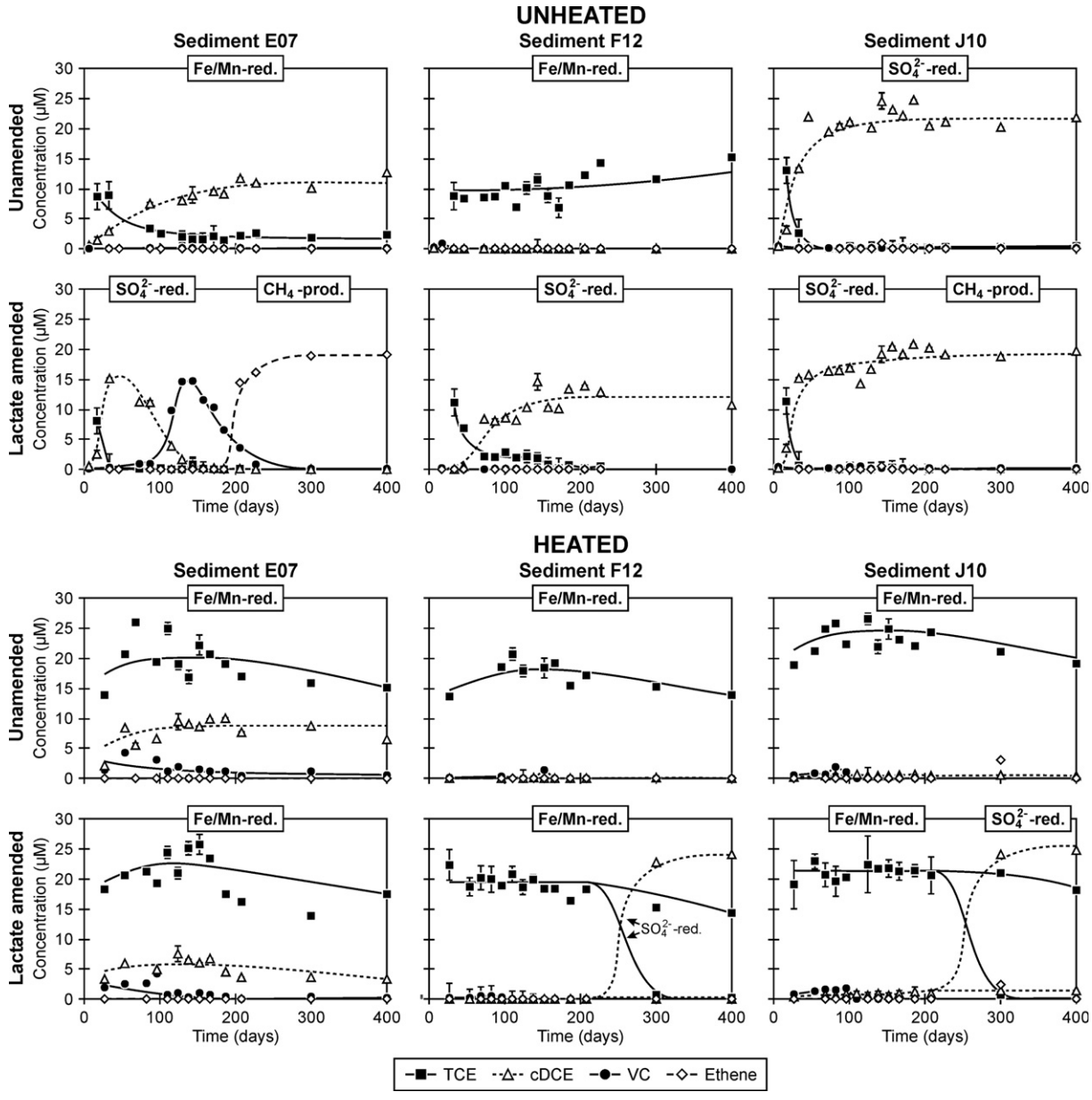

Fig. 2. Concentration of chlorinated ethenes and redox conditions in unheated and heated microcosms. All data points represent average values from duplicate or triplicate bioassays and bars show standard deviations. However, heated and lactate amended microcosms from F12 and J10, after 200 d are *not* shown as average, but as the result from each microcosm (i.e. dechlorination stalled at TCE in one replicate and was reduced to *c*DCE in the other replicate).

dechlorination in this study. Unheated and lactate-amended microcosms demonstrated complete dechlorination to ethene with sediment from location E07, and *c*DCE production with sediment from location F12 and J10. The redox parameters indicated Fe/Mn-reducing conditions in unamended microcosms with sediment from locations E07 and F12, and SO_4^{2-}-reducing conditions in microcosms with sediment from location J10 (Table 2). In all cases, the redox conditions became more reduced upon lactate amendment: CH_4 was produced in E07 and J10 sediment microcosms, and SO_4^{2-} was reduced in F12 sediment microcosms. Overall, unheated microcosms thus demonstrated partial dechlorination to TCE/*c*DCE when unamended and *c*DCE/ethene production when biostimulated with lactate.

The aqueous concentration of chlorinated ethenes in this study accounted for less than 1% of the available electron acceptors (Friis, 2006). This concentration was used to simulate levels observed at contaminated sites (approximately 20 μM TCE) and is lower than reported in previous studies (Fennell et al., 1997). Hence, the concentration of chlorinated ethenes used in this study should not unrealistically have favored growth of dechlorinating organisms over other microbes.

3.2. Dechlorination in heated microcosms

TCE was not dechlorinated in unamended and heated microcosms with sediment from all three locations (Fig. 2). In the heated microcosms with sediment from location E07, *c*DCE was present before TCE was added at higher concentrations than observed in the unheated microcosms. Abiotic hydrolysis of TCE seems unlikely since TCE dechlorination to *c*DCE has a half life reaction of 2×10^4 d^{-1} at 100 °C (Jeffers et al., 1989). Rather, the high initial concentrations of *c*DCE in the heated samples represent thermal desorption because *c*DCE was not present in unheated microcosms. After lactate addition, no dechlorination was observed in heated microcosms with sediment from location E07 (Fig. 2). However, *c*DCE production was observed after 200–300 d in one of two replicate microcosms with sediment from locations F12 and J10 (Fig. 2). The redox conditions remained Fe/Mn-reducing for at least 400 d in all heated microcosms except one lactate-amended microcosm with sediment from location F12 and two lactate-amended microcosms with sediment from location J10. The latter all became SO_4^{2-}-reducing (Table 2).

In summary, dechlorination of TCE was inhibited in heated, unamended microcosms and did not proceed past *c*DCE degradation in heated lactate-amended microcosms, suggesting a lack of active dechlorinating microorganisms including *Dehalococcoides*. A companion study has shown that during heating, thermal destruction of organic carbon results in increased dissolved organic carbon (DOC) concentrations that could be a source of electron donors for dechlorination (Friis et al., 2005). Results from this study (no dechlorination in heated, unamended microcosms) either suggest that, in heated microcosms, DOC released during the thermal treatment was not available to dechlorinating bacteria, or that the activity of dehalorespirers was too low.

3.3. PCR detection of specific dechlorinating microorganisms

The primers used in this study were selected to detect bacteria which are capable of either complete dechlorination (i.e. *Dehalococcoides*) or partial dechlorination to *c*DCE (i.e. *Geobacter*, *Dehalobacter,* and *Sulfurospirillum*) (Duhamel and Edwards, 2006; Grostern and Edwards, 2006). All control samples (including DNA from a mixed culture containing dechlorinators) were positive and the blanks (PCR reagents only) were negative on all gels used in this study. *Dehalococcoides*, *Dehalobacter,* and *Sulfurospirillum* were present in all unheated microcosms, and *Geobacter* was only detected in unheated microcosms with sediment from location E07 (Table 4). Following heat treatment, no *Dehalococcoides* were detected in any of the samples. *Dehalobacter* were detected only in lactate-amended microcosms with sediment from location F12 after 150 d of incubation. *Geobacter* were detected only in heated and lactate-amended microcosms with sediment from location J10. Interestingly, *Sulfurospirillum* were present in all heated and unheated sediments, suggesting that a thermo-tolerant member of this genus was ubiquitous at this site. Overall, these results demonstrated that *Dehalococcoides*, *Geobacter*, and *Dehalobacter* were detected in fewer microcosms after heating compared to unheated microcosms.

Since nested PCR is a non-quantitative method, a 'weak' signal does not necessarily imply that fewer bacteria are present but could indicate a signal from undergraded DNA. For example, in unheated microcosms with sediment from all three locations, a signal for *Dehalococcoides* was detected in unamended microcosms whereas the signal was weak/absent in lactate-amended microcosms. Yet, addition of lactate clearly promoted reductive dechlorination (Fig. 2). In this case, the *Dehalococcoides* signal detected in unamended microcosms may have arisen from undegraded genomic DNA of inactive cells.

3.4. Comparison of the extent of dechlorination, redox conditions, hydrogen concentrations, and the presence of dechlorinating microorganisms

Complete dechlorination to ethene requires (1) electron donors for dechlorination, commonly H_2 (Fennell et al., 1997; Löffler et al., 1999), (2) reducing conditions, (3) dechlorinating microorganisms, and (4) chlorinated electron acceptors. In this study, excess H_2 was present in all heated samples after 150 d of 10^3–10^6 nM. These elevated H_2 concentrations were observed both in unheated and heated microcosms at the beginning of the experiment

Table 2
Aqueous concentrations of redox parameters in microcosms at start and after 200 and 400 d of incubation

	Location E07						Location F12						Location J10					
	Unheated			Heated			Unheated			Heated			Unheated			Heated		
Days	Start	200	400	Start	200	400	Start	200	400	Start	200	400	Start	200	400	Start	200	400
Unamended microcosms																		
Mn_{diss}	0.05	0.10	5.04	0.02	0.07	3.03	<	0.03	0.84	<	<	0.49	0.06	0.13	7.50	<	0.03	1.91
Fe^{2+}	0.03	0.06	<	0.02	0.07	0.06	<	<	0.02	<	<	<	0.07	0.21	0.25	<	0.02	0.02
SO_4^{2-}	0.09	0.11	0.09	0.10	0.12	0.08	0.08	n.a.	0.04	0.05	0.08	0.10	0.08	<	<	0.06	0.08	0.12
CH_4	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<
H_2	10^2	0.1	1.3	10^2	10^3	10^3	10^2	0.38	1.38	10^2	45.8	0.78	10^4	0.12	1.45	10^4	10^5	2.24
Lactate	<	<	<	<	<	<	<	<	<	<	<	<	0.03	<	<	<	<	<
Acetate	0.06	0.02	<	0.17	0.03	0.04	0.06	0.04	<	<	<	<	0.03	0.02	<	0.05	0.03	0.53
Lactate amended microcosms																		
Mn_{diss}	0.07	0.18	7.25	0.02	0.08	4.76	<	0.08	n.a.	<	0.02	1.96	0.08	0.15	10.1	<	0.05	3.21
Fe^{2+}	0.19	1.23	0.08	0.02	0.29	0.29	<	0.08	0.08	<	<	<	0.09	0.27	0.23	<	0.04	0.15
SO_4^{2-}	0.05	<	<	0.11	0.10	0.08	0.04	<	<	0.05	0.06	0.05	0.07	<	<	0.05	0.04	<
CH_4	<	0.1	7.1	<	<	<	<	<	<	<	<	<	<	<	4.7	<	<	<
H_2	14	7.5	1.6	10^2	10^3	1.1	10^2	1.56	0.44	10^2	10^2	10^2	10^4	48.5	1.5	10^4	10^5	10^4
Lactate	1.48	<	<	2.88	<	<	2.46	<	<	2.59	2.69	2.16	2.42	<	<	2.50	3.54	0.72
Acetate	0.22	0.10	0.03	0.17	3.63	4.32	0.06	1.25	1.25	0.05	0.08	0.49	0.06	4.36	3.20	0.06	0.16	1.97

All parameters are given in mM. < indicate below detection limit of: 0.01 mM Mn_{diss}, 0.015 mM Fe^{2+}, 0.001 mM SO_4^{2-}, 0.03 mM CH_4, and 0.01 mM lactate and acetate. All NO_3^- concentrations remained below the detection limit of 0.003 mM. Start is at day 0 in unheated samples and day 14 in heated samples, except sampling of fatty acids which was at day 6 in unheated and day 27 in heated microcosms.

Table 3
Presence of chlorinated ethenes in samples prior to setup of microcosms

Location	Sediment (μmol/kg)			Groundwater (μM)		
	TCE	*c*DCE	VC	TCE	*C*DCE	VC
E07	15.02	0.72	0.5	20.6	21.7	0.48
F12	0.38	<	<	2.28	<	<
J10	1.27	0.95	0.6	0.48	0.74	<

< indicate below detection limits of: 0.02 μmol/kg TCE and *c*DCE, 0.07 μmol/kg VC. Sediment samples used in these microcosms were analyzed prior to setup as μmol/kg sediment slurry. Sediment samples were collected in August 2003 prior to setup of microcosms and analysed as described in Materials and Methods within 10 d of sampling. Groundwater was sampled from boreholes next to the sediment sampling locations (E07, F12, and J10) and analyzed in November 2003. The concentrations represent average levels.

and probably stem from the experimental set-up despite several attempts to minimize initial H_2 concentrations (see Section 2). However, after 200–400 d, the levels decreased to 1–2 nM H_2 in lactate-amended E07, unamended F12, and unamended J10 microcosms, but remained elevated in the remaining heated microcosms (Table 2). In mixed cultures and field studies, levels of 0.6–0.9 nM H_2 for PCE and TCE reduction, 0.1–2.5 nM H_2 for *c*DCE reduction, and 2–24 nM H_2 for VC reduction have been reported (Yang and McCarty, 1998; Lu et al., 2001). The availability of electron donors, specifically H_2, should therefore not have been limiting for dechlorination in all heated microcosms. In unheated microcosms, H_2 consumption occurred faster within 30–150 d to 1–10 nM and further to 1–2 nM after 400 d in unamended microcosms (Table 2). The H_2 levels in unheated microcosms were therefore at or above the level commonly observed during *c*DCE reduction, although complete dechlorination to ethene seems to have required additional electron donor (i.e. was restricted to lactate-amended microcosms with sediment from location E07) before approximately 300 d of incubation.

In unheated, unamended and lactate-amended microcosms significant dechlorinating activity of TCE to *c*DCE was observed. However, complete dechlorination to ethene occurred only in unheated and lactate-amended microcosms with sediment from location E07, where conditions were appropriate for complete dechlorination to occur: methanogenic conditions prevailed and sufficient electron donor and *Dehalococcoides* were present. In unheated microcosms with sediment from locations F12 and J10, *Dehalococcoides* were present but the conditions were largely SO_4^{2-}-reducing and dechlorination stalled at *c*DCE production. Here, other electron acceptors may have been favored over the chlorinated ethenes, or else the *Dehalococcoides* detected were not capable of complete dechlorination (Bunge et al., 2003). Electron donors for dechlorination were present as acetate (He et al., 2002) in all unheated lactate-amended microcosms after 400 d of incubation (Table 2). Very few *Dehalococcoides* were present overall, as revealed by the need to use nested PCR to detect them at all. Thus, various factors may have resulted

Table 4
Presence of dechlorinators evidenced by nested PCR and summary of dechlorination

		Days	Location E07					Location F12					Location J10				
			Dehaloco-ccoides	*Geo-bacter*	*Dehalo-bacter*	*Sulfuro-spirillum*	Chlorinated ethene	*Dehaloco-ccoides*	*Geob-acter*	*Dehalob-acter*	*Sulfuro-spirillum*	Chlorinated ethene	*Dehaloco-ccoides*	*Geob-acter*	*Dehalo-bacter*	*Sulfuro-spirillum*	Chlorinated ethene
Unheated	Unamended	10	+	(+)	+	+	TCE	+	–	+	+	TCE	(+)	–	+	+	TCE
		150	+	+	+	+	*c*DCE	(+)	–	+	+	TCE	+	–	+	+	*c*DCE
	Lactate	150	(+)	(+)	+	+	VC	–	–	(+)	+	*c*DCE	(+)	–	(+)	+	*c*DCE
Heated	Unamended	10	–	–	–	(+)	TCE	–	–	–	+	TCE	–	–	–	+	TCE
		150	–	–	–	(+)	TCE	–	–	–	+	TCE	–	–	+	+	TCE
	Lactate	150	–	–	–	+	TCE	–	+	–	+	TCE	–	–	(+)	+	TCE

+ indicate a signal, (+) a weak signal, and – no signal on the gel. The presence of dechlorinators was investigated in DNA extracted from sediment slurry obtained from anaerobic sacrificed microcosms.

in little dechlorinating activity of *c*DCE to ethene in unheated microcosms, including low concentrations of dechlorinating microorganisms and electron acceptors (chlorinated ethenes), inability of *Dehalococcoides* to dechlorinate *c*DCE to ethene as well as competition from Fe-, Mn-, and SO_4^{2-}-reducers and methanogens for scarce nutrients and, for example, electron donors.

In the heated samples, TCE was only transformed in 2 out of 12 microcosms and it stalled at *c*DCE in both microcosms. This lack of TCE dechlorination could be explained by the overall low bacterial activity because the redox conditions remained Fe/Mn-reducing in unamended sediments. Correspondingly, dechlorinating microorganisms were detected in fewer heated than unheated microcosms. Nevertheless, the presence of *Sulfurospirillum* in all sediments and the occurrence of *Geobacter* in F12 microcosm and *Dehalobacter* in J10 microcosm demonstrated that some dechlorinating organisms survived the heating. Nested PCR is non-quantitative and can therefore not be used to determine an increased abundance of *Geobacter* after heating in lactate-amended microcosms with sediment from location F12. Furthermore, other types of bacteria have demonstrated the capability of degrading TCE to *c*DCE and not all of these were tested for in this study. For example, *Clostridium bifermentans* (Chang et al., 2000) and *Desulfitobacterium metallireducens* (Finneran et al., 2002) can also form spores and may thus withstand elevated temperatures. The survival of microorganisms capable of partial dechlorination of TCE to *c*DCE was confirmed in this study after approximately 200 d of incubation although this transformation occurred in fewer heated than unheated microcosms. The absence of dechlorination past *c*DCE in heated microcosms and the decrease in number of microcosms with *Dehalococcoides* suggested that these microorganisms were rendered inactive by heating. However, since ethene only was produced in microcosms with sediment from one location, an equally likely explanation would be that the site *Dehalococcoides* organisms were not proficient at *c*DCE dechlorination or simply present in too little numbers.

3.5. Perspectives of this study

These microcosm studies demonstrate that dechlorination of TCE to *c*DCE was limited or postponed after heating sediment and groundwater from the Ft. Lewis site in closed microcosms. Nevertheless, some dechlorinating organisms survived heat treatment, although they required a long recovery phase. Laboratory experiments were conducted under conditions whereby the heating was strictly controlled. Thereby the effects of non-uniform temperatures and pressures (possibly allowing more bacteria to survive in cooler areas) as well as spatial variation in biogeochemistry are ruled out. Our results suggest that if extensive dechlorination of TCE occurs after a thermal treatment in the field, it may stem from an influx of groundwater containing bacteria or from areas that have not been heated to 100 °C. Conversely, limited dechlorination of TCE to *c*DCE may occur in areas that have been heated to 100 °C without inflowing groundwater transporting microorganisms, electron acceptors, nutrients, etc. Post-thermal biodegradation in a remediated source zone may thus be possible by repopulation of indigenous bacteria with groundwater, assuming that microorganisms capable of complete dechlorination are present at the site, or by bioaugmentation, where the dechlorinating microorganisms are added.

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